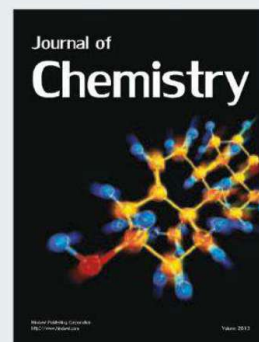
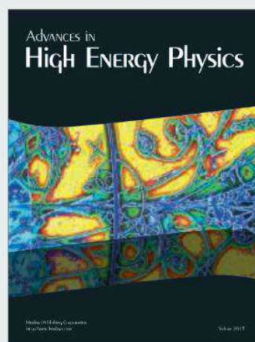
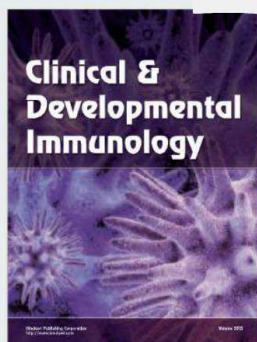
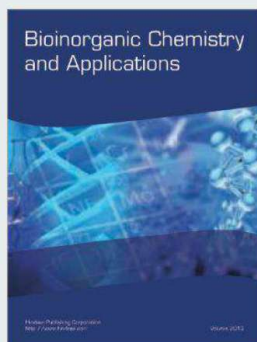
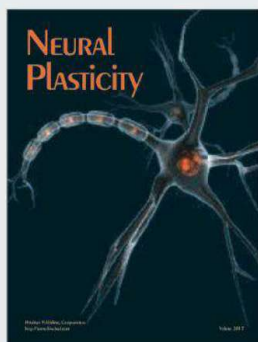
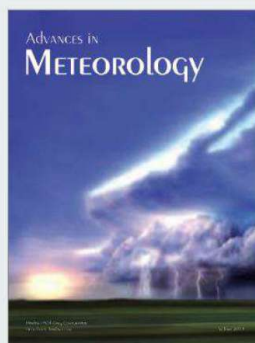
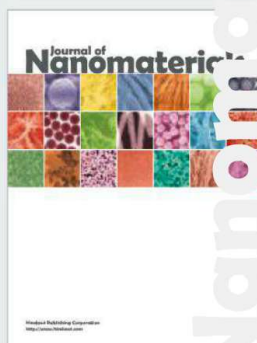
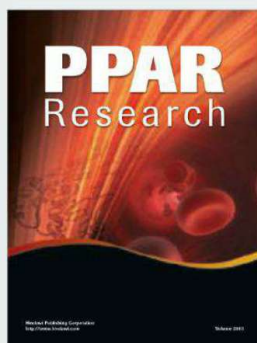
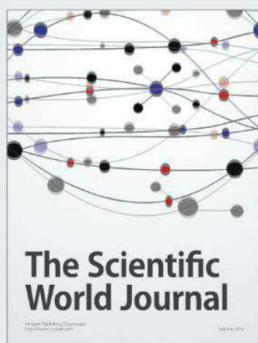
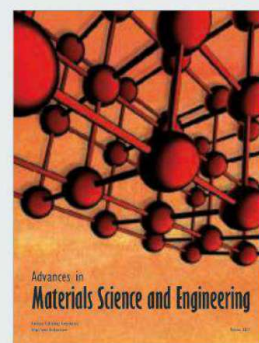
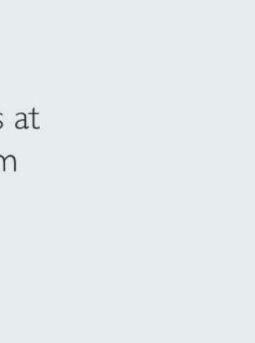
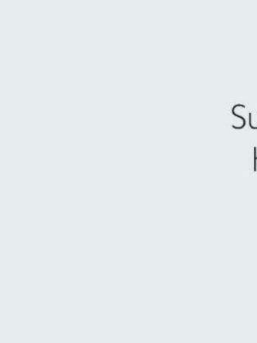
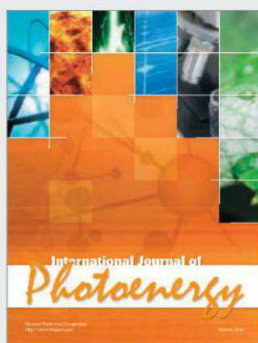
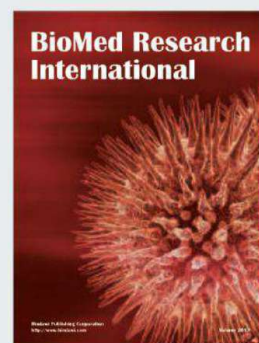
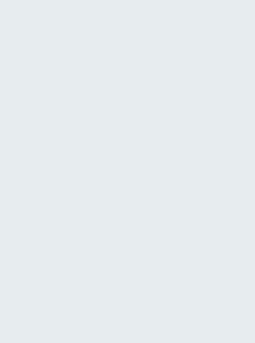
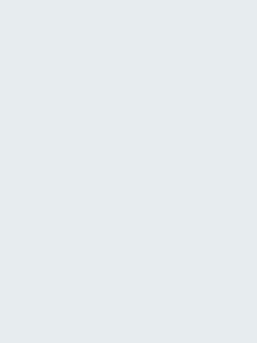
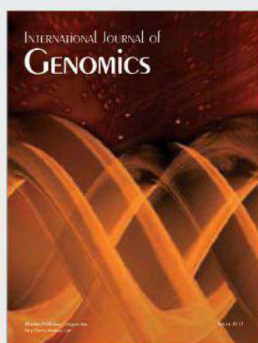
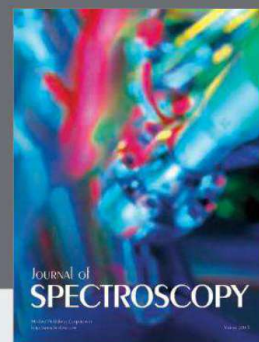
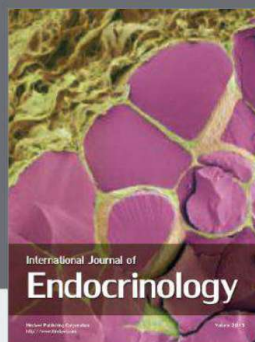
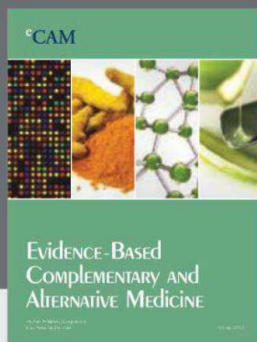
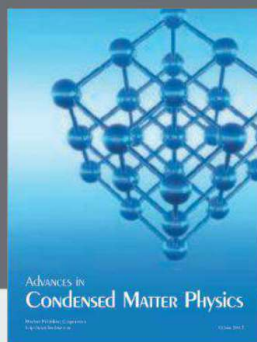
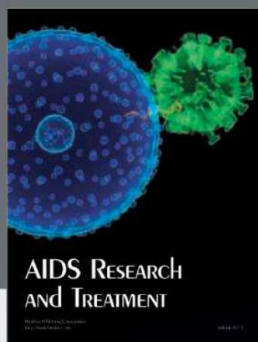


19 April 2013 | \$10

Science

Grand Challenges
in Science Education



Hindawi

Submit your manuscripts at
<http://www.hindawi.com>

More affordable BD Research Reagents

Lower pricing on over one thousand high-quality products.

Welcome to
affordable
a more colorful
world.SM

Lower pricing means you don't have to sacrifice quality.

Enjoy the best combination of product quality and value when you select BD Biosciences multicolor flow cytometry reagents.

For over 25 years BD PharmingenTM brand reagents have been developed and manufactured with strict controls and high standards to ensure high lot-to-lot consistency and performance on BD flow cytometers.

Our ever expanding portfolio is the most cited on the market and includes rare combinations of antibodies and fluorochromes that let you use BD reagents across your entire multicolor panel. Recent portfolio additions include the innovative



BD

Helping all people
live healthy lives

BD HorizonTM Brilliant VioletTM dyes which are 5–10X brighter than conventional dyes to assist the resolution of rare or dim populations.

All BD Biosciences reagents are backed with expert technical support and a variety of tools and information to enable you to get the most from your multicolor panels and help speed your research. Now, new pricing makes high quality and reliability more affordable.

You may also be eligible for a more attractive institutional discount—contact your BD Biosciences sales representative to find out more at bdbiosciences.com/reagents

The History and Achievements of the School of the Gifted Young

An Education Fit for China's Finest

The School of the Gifted Young (SGY) at the University of Science and Technology of China has been educating China's brightest youths for over 30 years. The school offers a unique opportunity for those who are ready for higher education, but are too young to enter a normal university. This forward-thinking institution keeps class sizes small and has a rigorous admittance process to maintain its impeccable standards of education.

In 1974, during the tumult of the Cultural Revolution, Professor Tsung-Dao Lee was granted a rare dialogue with Chairman Mao Zedong, then-leader of the Communist Party. As a Nobel laureate in physics at the young age of 31, Lee's dream was to reform the education system to allow brilliant young minds access to higher education as soon as they were ready, irrespective of age. After writing letters to the relevant powers in the central government advocating education for the especially gifted, Lee oversaw the first class of 28 youths at the University of Science and

Technology of China (USTC) in 1978. Among them was a young man with razor-sharp intellect named Ya-Qin Zhang. Now chairman of Microsoft's Asia-Pacific operations, Zhang was 12 years old when he first enrolled in SGY.

Since the late 1970s, thousands of bright young people like Zhang have prospered in the college. Situated in the east campus of USTC in Hefei, Anhui Province, overlooking an expanse of tree-shaded grass, the school's many notable alumni greet visitors from portraits as they enter the lobby. Among them is a picture of Zhang himself, whose achievements, while remarkable, are equaled by many of his SGY peers. Over 2,000 prodigies have graduated from SGY thus far; among them are some of the most influential names in China and beyond.

"Our school's education system is superior for undergraduate students," says Professor Yang Chen, the school's executive dean. "We hope it gives the gifted young the chance to reach their potential and find success."



SGY Freshmen Attending a Class Lecture (2011)

Recruiting the Best and the Brightest

Getting a place at the school is, as expected, highly competitive. Compared with students at China's elite universities, such as Fudan or Tsinghua University, Chen estimates that 50% of the SGY scholars would be considered to have "excellent" academic performance. (When compared with China's standard universities, Chen says 90% would be classed as such.)

Currently, there are three types of admission processes at SGY, which depend on a student's age. The "Special Class for the Gifted Young," for which the school was founded, recruits students under the age of 16. Each year around 3,000 remarkably bright students apply to this class. The admissions process includes a review of the students' university entrance exam scores as well as campus tests and one-on-one interviews. Starting in 1985, a second class type was created, the "00 Class," to select the most promising students from USTC's newly enrolled freshman, who

are typically 18 or older. The third type, the "Innovation Class," began in 2010 and uses an entirely different admissions process. It is open to Grade 11 students, aged 16 to 17, who are recommended by their high school headmasters and pre-evaluated before they take the *gaokao*, or college entrance exam. SGY's unique admission processes increase the diversity of the student population and provide exceptional students several different ways to join the school.



A Distinctive Curriculum

A big draw for students and parents alike is SGY's unique teaching model. Since it was founded, SGY has crafted a high-requirement curriculum and flexible educational system that sets it apart from other academic institutions. One of the biggest differences—and one that is particularly suited to younger minds—is the delay in choosing a major. In their first year at SGY, students take a broad set of courses encompassing math, physics, chemistry, engineering, and English language. Only at the end of their freshman year are they asked if they are ready to choose a major.

"We encourage students to study what they're interested in," says Chen. "Not to think 'which major will make money,' but to find their motivation and passion. If the student still doesn't know what they want to major in, they can create an 'individualized study plan.'" This seemingly simple accommodation allows sufficient time for the students to discover their true interest—something that can be intimidating or perplexing in the crowded high school education system, when most students in China decide. "SGY values students' individuality and expects students to achieve their full potential," Chen adds.

This transition year enables students to enter into regular university life with confidence and ease. Chen explains that when SGY students merge into the greater student body at USTC, they are often in the top 10% of their classes, despite the age difference. When the prominent alumni, Xiaowei Zhuang, who is now a professor at Harvard University, applied to the graduate school at Berkeley there was a misunderstanding with her academic transcript. "Berkeley called us to confirm, because her score was 100—full marks—and they thought it was fake," Chen laughs, adding: "She was one of the best students and younger than the other applicants by three years."

The SGY students' relative youth does not pose a barrier for their academic performance, however. SGY's staff provides counseling to help students smoothly adapt to college life, while a busy 60-hour week keeps the students engaged. Though teaching standards are high, it is the students themselves who push their achievements beyond expectations, Chen says. "All the students feel honored to be here," he says, adding that because the students have skipped years in high school, their confidence is reinforced. "They believe that because our school is special, they should work hard. They are self-disciplined and try their best," he says.



Student Champions in the 2012 USTC Sports Meeting



Group Discussion During an Open Laboratory (2010)



SGY Building

Accomplished Alumni

Though the School of the Gifted Young is still relatively youthful itself—having been founded just 34 years ago—turning bright minds into leaders is something in which the school has long excelled. A brief glance at the achievements of former students, many of whom are prominent figures in diverse fields, attests as much. Of the 2,000 alumni (90% of whom went on to achieve either Master's or Ph.D. degrees), over 200 are now professors at the world's most accomplished institutions, including Harvard University, Yale University, the Massachusetts Institute of Technology, Princeton University, the University of Birmingham and, also, China's finest schools such as Beijing's Tsinghua University and Shanghai's Fudan University.

Former SGY pupils are frequently the recipients of the most prestigious awards in science and technology fields. In 2012, alumni Xiaowei Zhuang (of Harvard University) and Ligu Luo (of Stanford University) were elected as academicians of the U.S. National Academy of Sciences. Other SGY alumni have become fellows at IEEE, the Optical Society of America, and the American Physical Society and have received early career awards such as the Alfred P. Sloan Fellowship, the National Science Foundation Career Award, and the Presidential Early Career Award for Scientists and Engineers (PECASE).

Moreover, many alumni have become leaders in industry, commerce, and finance. For example, Yuanlin Guo, who enrolled in 1978, is today vice president of Tsinghua Unisplendour Corporation Limited. Twenty percent of SGY's alumni are today employed on global financial trading floors, including over a hundred on Wall Street alone.

Creating the Leaders of the Future

To attain positions of such prestige, young people need more than just robust intellect. This is something Yang Chen and the teachers at SGY understand well. "Since I started here, my goal has been to prepare students to become the future leaders in different fields and professions," says Chen. "The key to becoming a leader is learning how to work in teams. The most valuable part of SGY is the students themselves. We expect the students to teach and learn from each other and to work in close cooperation."

To assist with the students' social development and to ensure they receive a well-rounded education, SGY offers a number of opportunities outside the academic realm. Students are highly encouraged to participate in many types of outreach and volunteering programs, including helping children with disabilities, teaching in high schools, and protecting the environment, so they can learn about social responsibility. "During winter and summer breaks, the students volunteer in rural parts of China where educational resources are deficient," says Chen. "To become the leaders of the future, students need to gain a better understanding of social issues, accept heavier responsibilities, and return more to society."

In the future, Chen and colleagues are determined to maintain a high standard of education and have their teaching methods naturally evolve to fit the times. "Going forward, we would like to learn how to better accommodate the students' needs and reform the edu-

cation system," Chen says. "Students today learn a lot from the Internet, which is very different from when I was a student. So many staff members, including myself, are exploring new ways to teach this next generation. It used to take 20 years for a new generation to form, now I think it is closer to five years," he adds. "Things are different today." Though that may be true, there is no doubt that SGY will—just like the students themselves—be well ahead of the curve.



18-Year-Old Students Attending the 2010 "Adult Ceremony" in Jiangdu, China

For more information, visit
www.Q5PCR.com

Fidelity at its finest.

Q5[®] High-Fidelity DNA Polymerase

Q5 High-Fidelity DNA Polymerase sets a new standard for both fidelity and performance. With the highest fidelity amplification available (>100X higher than *Taq*), Q5 DNA Polymerase results in ultra-low error rates. Its unique buffer system provides superior performance for a broad range of amplicons, regardless of GC content. Available in master mix and hot start formulations, Q5 DNA Polymerase represents the finest in fidelity.

ALSO AVAILABLE: Optimized NEBNext[®] formulation for next generation sequencing library amplification

Mandarin Ducks (*Aix galericulata*) are frequently featured in Chinese art and are regarded as a symbol of fidelity.

Robust amplification even with high GC amplicons



Amplification of two human genomic amplicons of mid to high GC content. All reactions were conducted using 30 cycles of amplification and visualized by microfluidic LabChip[®] analysis. All polymerases were cycled according to manufacturer's recommendations. For the 78% GC amplicon, GC Buffers or enhancers were used when supplied with the polymerase.

A = Q5[®] High-Fidelity DNA Polymerase (NEB)
B = Phusion[®] High-Fidelity DNA Polymerase (NEB)
C = KOD DNA Polymerase (EMD)
D = PfuUltra[™] High-Fidelity DNA Polymerase (Agilent)

PHUSION[®] is a registered trademark and property of Thermo Fisher Scientific. Phusion[®] DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. PFUULTRA[™] is a trademark of Agilent Technologies, Inc. LABCHIP[®] is a registered trademark of Caliper Life Sciences, part of Perkin Elmer, Inc. NEBNext[®] and Q5[®] are registered trademarks of New England Biolabs, Inc.

SPECIAL SECTION

Grand Challenges in Science Education

INTRODUCTION

- 290 Plenty of Challenges for All

NEWS

- 292 Transformation Is Possible if a University Really Cares
>> *Science Podcast*

REVIEWS

- 297 The Challenge of Education and Learning in the Developing World
M. Kremer et al.
- 300 Understanding Neurocognitive Developmental Disorders Can Improve Education For All
B. Butterworth and Y. Kovas
- 305 Physical and Virtual Laboratories in Science and Engineering Education
T. de Jong et al.
>> *Science Podcast*
- 310 Professional Development for Science Teachers
S. M. Wilson

- 314 Outside the Pipeline: Reimagining Science Education for Nonscientists
N. W. Feinstein et al.
>> *Science Podcast*
- 317 Generating Improvement Through Research and Development in Education Systems
M. S. Donovan
- 320 Proficiency in Science: Assessment Challenges and Opportunities
J. W. Pellegrino

PERSPECTIVES

- 309 Teacherpreneurs: A Bold Brand of Teacher Leadership for 21st-Century Teaching and Learning
B. Berry
- 313 A Business View on U.S. Education
R. Stephens and M. Richey
- >> Editorial p. 249; Education Forums pp. 276 and 278; Science Signaling, Science Careers, and more at www.sciencemag.org/special/education2013

EDITORIAL

- 249 Prioritizing Science Education
Bruce Alberts
>> *Grand Challenges in Science Education* section p. 290

NEWS OF THE WEEK

- 254 A roundup of the week's top stories

NEWS & ANALYSIS

- 257 Request Would Let Science Rebound From Sequester
- 258 Wild Cards Remain After Proposed Reshuffle of STEM Education

- 261 Archaeologists Say the 'Anthropocene' Is Here—But It Began Long Ago
- 262 Kepler Snags a Super-Earth-Size Planet Squarely in a Habitable Zone
>> *Science Express Report* by *W. J. Borucki et al.*
- 263 Rare Cancer Successes Spawn 'Exceptional' Research Efforts
- 265 Survey of Peers in Fieldwork Highlights an Unspoken Risk

NEWS FOCUS

- 266 Trachea Transplants Test the Limits
- 269 Chasing Ants—and Robots—to Understand How Societies Evolve
The Private Lives of Ants
>> *Science Express Report* by *D. P. Mersch et al.*

LETTERS

- 272 Drought and China's Cave Species
S.-S. Shu et al.
- Little Emperors Pose Behavioral Challenges
L. Cameron et al.
- Preventing Prejudice in Genome Profiling
M. Maruthappu and A. E. Finlayson
- 273 CORRECTIONS AND CLARIFICATIONS
- 273 TECHNICAL COMMENT ABSTRACTS

BOOKS ET AL.

- 274 The Earthquake Observers
D. R. Coen, reviewed by G. C. Beroza
- 275 Edinburgh International Science Festival
reviewed by D. Dixon and E. Straughan
>> *Science Podcast*

EDUCATION FORUMS

- 276 Opportunities and Challenges in Next Generation Standards
E. K. Stage et al.
- 278 Driven by Diversity
J. Ferrini-Mundy
>> *Grand Challenges in Science Education* section p. 290

PERSPECTIVES

- 279 3D Mapping in the Brain
C. Barry and C. F. Doeller
>> *Reports* pp. 363 and 367
- 280 Climate's Dark Forcings
M. O. Andreae and V. Ramanathan

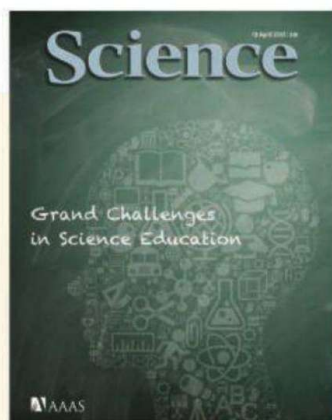
CONTENTS continued >>

ON THE WEB THIS WEEK

>> **Special Issue Video**
Watch a video about improving education in the developing world at www.sciencemag.org/special/education2013.

DEPARTMENTS

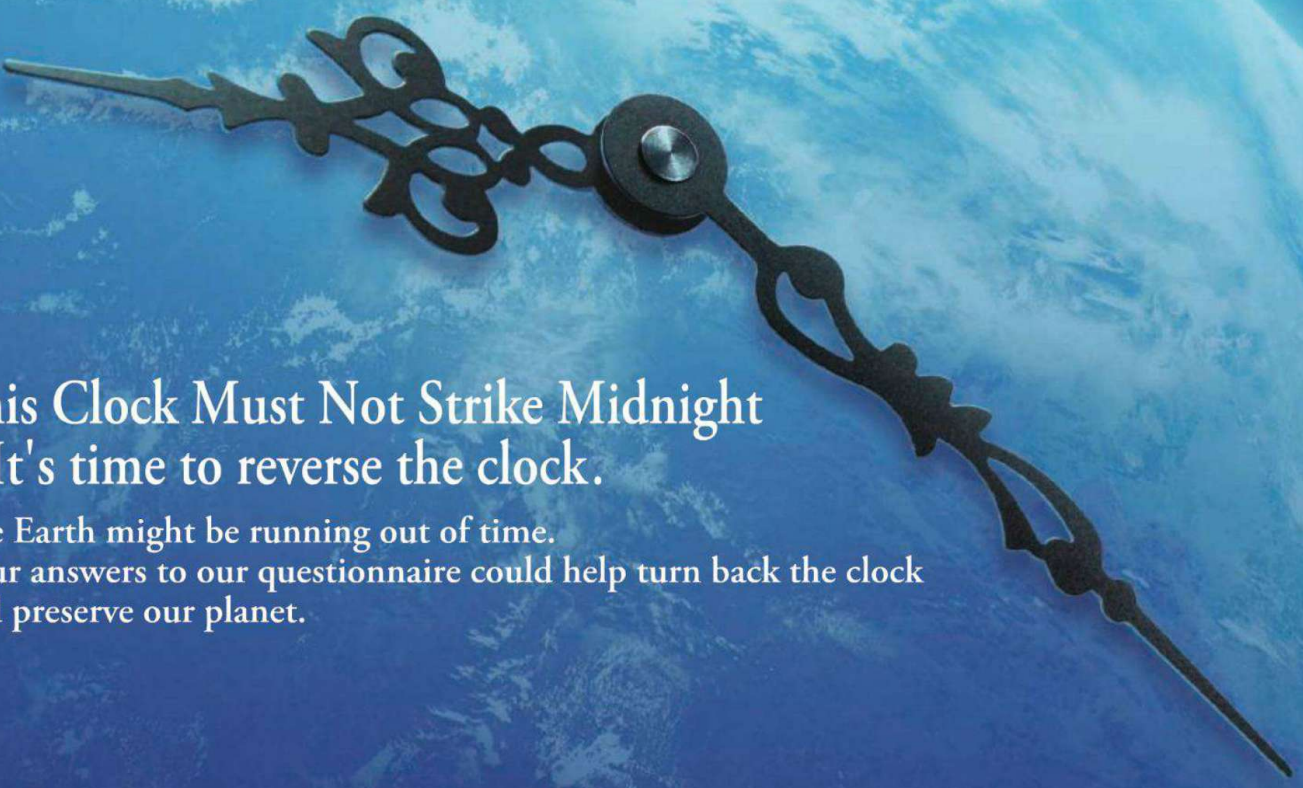
- 247 This Week in Science
- 250 Editors' Choice
- 252 Science Staff
- 382 New Products
- 384 Science Careers



COVER

The 21st-century scientific workforce needs new skills and knowledge to keep pace with ever-changing technology. Larger, more diverse student populations clamor for access to knowledge. All global citizens, whether in a doctor's office or polling booth, must be better informed. All of these needs call for expanded, improved science education. In this special issue, we have invited experts to discuss the most important challenges facing science education. See the special section beginning on page 290.

Images: Thinkstock

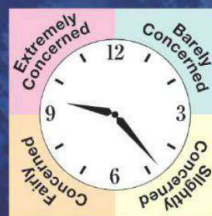


This Clock Must Not Strike Midnight — It's time to reverse the clock.

The Earth might be running out of time.
Your answers to our questionnaire could help turn back the clock
and preserve our planet.

Questionnaire Respondents Wanted

The **Environmental Doomsday Clock** displays the level of anxiety felt by respondents about the ultimate fate of the Earth, with 12:00 representing the highest level of concern—in effect—that time has run out.



This survey is open to all who are concerned about maintaining a healthy and sustainable diversity of life on Earth. Your answers to the questionnaire will help raise environmental awareness around the world, and we are grateful to you for taking the time to give a considered response.

Register to receive the questionnaire at : www.af-info.or.jp

- 282 Heterochronic Genes Turn Back the Clock in Old Neurons**

P. Nix and M. Bastiani

>> Report p. 372

- 283 Polarization Traffic Control for Surface Plasmons**

A. E. Miroshnichenko and Y. S. Kivshar

>> Reports pp. 328 and 331

- 284 Great Apes and Zoonoses**

P. M. Sharp et al.

- 286 Pursuing Near-Zero Response**

N. Engheta

- 287 Fire in the Ocean**

C. A. Masiello and P. Louchouart

>> Report p. 345

RESEARCH ARTICLE

- 324 Pervasive Externalities at the Population, Consumption, and Environment Nexus**

P. S. Dasgupta and P. R. Ehrlich

Challenges posed by the economic consequences of population growth and consumption require collective action.

REPORTS

- 328 Near-Field Interference for the Unidirectional Excitation of Electromagnetic Guided Modes**

F. J. Rodríguez-Fortuño et al.

Near-field interference can be used to control the directional propagation of electromagnetic excitations.

- 331 Polarization-Controlled Tunable Directional Coupling of Surface Plasmon Polaritons**

J. Lin et al.

Control over the generation and propagation direction of light-induced surface plasmons in a thin metal film is demonstrated.

>> Perspective p. 283

- 334 External Quantum Efficiency Above 100% in a Singlet-Exciton-Fission-Based Organic Photovoltaic Cell**

D. N. Congreve et al.

Single photons are shown to propel more than one carrier in a carbon-based solar cell.

- 337 Multicompartment Mesoporous Silica Nanoparticles with Branched Shapes: An Epitaxial Growth Mechanism**

T. Suteewong et al.

A one-pot synthesis method furnishes mesoporous silica nanoparticles with both cubic and hexagonally structured compartments.

- 341 Reorganization of Southern Ocean Plankton Ecosystem at the Onset of Antarctic Glaciation**

A. J. P. Houben et al.

The Southern Ocean plankton ecosystem underwent an abrupt and profound reorganization in the earliest Oligocene.

- 345 Global Charcoal Mobilization from Soils via Dissolution and Riverine Transport to the Oceans**

R. Jaffé et al.

A larger-than-assumed fraction of charcoal produced by wildfires leaches out of soils and is transported to the oceans.

>> Perspective p. 287

- 347 Resilience and Recovery of Overexploited Marine Populations**

P. Neubauer et al.

Current fish harvests and low fish levels make fishery recovery improbable for most of the world's depleted stocks.

- 350 A KRAB/KAP1-miRNA Cascade Regulates Erythropoiesis Through Stage-Specific Control of Mitophagy**

I. Barde et al.

Protein- and RNA-based transcriptional regulation governs the removal of mitochondria during red blood cell differentiation.

- 353 The Helicase-Like Domains of Type III Restriction Enzymes Trigger Long-Range Diffusion Along DNA**

F. W. Schwarz et al.

A bacterial enzyme that cuts DNA uses a few adenosine triphosphates to allow it to scan across thousands of base pairs.

- 356 Structural Basis for Kinesin-1: Cargo Recognition**

S. Pernig et al.

The structure of a portion of a molecular motor complexed to a cargo peptide provides a close-up view of the interaction.

- 359 Actin-Propelled Invasive Membrane Protrusions Promote Fusogenic Protein Engagement During Cell-Cell Fusion**

K. Shilagardi et al.

An inducible *Drosophila* cell-fusion system reveals the interplay between cellular fusion proteins and actin-driven membrane remodeling.

- 363 Bat and Rat Neurons Differ in Theta-Frequency Resonance Despite Similar Coding of Space**

J. G. Heys et al.

Stellate cells in the entorhinal cortex of bats and rats show significant differences in their electrophysiological properties.

>> Perspective p. 279

- 367 Representation of Three-Dimensional Space in the Hippocampus of Flying Bats**

M. M. Yartsev and N. Ulanovsky

The spatial firing properties of neurons were recorded in bats during flight using a wireless neural-telemetry system.

>> Perspective p. 279



page 269



page 274

- 372 Developmental Decline in Neuronal Regeneration by the Progressive Change of Two Intrinsic Timers**

Y. Zou et al.

Reciprocal signals promote axon regeneration in young worms and repress axon regeneration in older worms.

>> Perspective p. 282

- 376 A Neural Marker of Perceptual Consciousness in Infants**

S. Kouider et al.

The brain mechanisms underlying conscious perception are already present in infancy and improve with age.

SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Periodicals Mail postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Copyright © 2013 by the American Association for the Advancement of Science. The title SCIENCE is a registered trademark of the AAAS. Domestic individual membership and subscription (S1 issues): \$149 (\$74 allocated to subscription). Domestic institutional subscription (S1 issues): \$990; Foreign postage extra: Mexico, Caribbean (surface mail) \$55; other countries (air assist delivery) \$85. First class, airmail, student, and emeritus rates on request. Canadian rates with GST available upon request, GST #1254 88122. Publications Mail Agreement Number 1069624. Printed in the U.S.A.

Change of address: Allow 4 weeks, giving old and new addresses and 8-digit account number. **Postmaster:** Send change of address to AAAS, P.O. Box 96178, Washington, DC 20090-6178. **Single-copy sales:** \$10.00 current issue, \$15.00 back issue prepaid includes surface postage; bulk rates on request. **Authorization to photocopy** material for internal or personal use under circumstances not falling within the fair use provisions of the Copyright Act is granted by AAAS to libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that \$30.00 per article is paid directly to CCC, 222 Rosewood Drive, Danvers, MA 01923. The identification code for Science is 0036-8075. Science is indexed in the Reader's Guide to Periodical Literature and in several specialized indexes.

NEW!



LightCycler®

Innovative. Intuitive. Accurate. *The LightCycler® 96 Real-Time PCR System*

- **Innovative optics for accurate results**

Simultaneously capture 96 wells of data for maximum well-to-well consistency.

- **Intuitive touchscreen interface and powerful data analysis software**

Speed time to results with an advanced yet easy-to-use system designed for both novices and experienced users.

- **Built on more than 15 years of real-time PCR excellence**

Rely on Roche's expertise in providing fast, flexible, and accurate real-time PCR systems that enable research breakthroughs.



**Scan to see
the video.**

Contact your Roche representative, call **800 262 1640**, or visit
www.lightcycler96.com for more information.

**For life science research only.
Not for use in diagnostic procedures.**

LIGHTCYCLER is a trademark of Roche.

Roche Diagnostics Corporation
Roche Applied Science
Indianapolis, Indiana



© 2013 Roche Diagnostics.
All rights reserved.

<< Controlling Light Propagation

Surface plasmons are light-induced collective electronic excitations in a metal that offer the possibility of manufacturing optoelectronic devices at nanometer scale. Before such shrinking can be achieved, the propagation direction and lifetime of the plasmonic excitations have to be controlled (see the Perspective by **Miroshnichenko and Kivshar**). **Rodríguez-Fortuño et al.** (p. 328) show how this is done using polarized light. Alternatively, using an array of metallic nanoantennae (in this case, slits) patterned into a thin gold film, **Lin et al.** (p. 331) present a further improvement on current plasmonic coupling schemes that has the potential to encode information contained in both the intensity and polarization of light.

Sticky Responses

A textbook example of an unaccounted-for consequence (externality) of commercial or industrial activity is the production of pollutants where neither the producer nor the buyer bears the cost of using common environmental resources. **Dasgupta and Ehrlich** (p. 324) offer a theoretical analysis of externalities in two other areas of modern life—human fertility and material consumption. For example, when fertility decline lags mortality decline, the consequence could be environmental crash, the likelihood of which is greater if the environmental effects of consumption or population growth are external to the market.

Tuning Mesopores

Porous materials are of interest for catalysis and filtration because the open channels lend themselves to separating materials or function. **Suteewong et al.** (p. 337) report on a method to make branched mesoporous silica nanoparticles that contain cubic (core) and hexagonally structured (branch) parts within one particle. Controlling the extent of the branched structure is achieved by tuning the concentration of additives in a simple, one-pot reaction system.

Dissolving Charcoal

Biomass burning produces 40 to 250 million tons of charcoal per year worldwide. Much of this is preserved in soils and sediments for thousands of years. However, the estimated production rate of charcoal is significantly larger than that of decomposition, and **Jaffe et al.** (p. 345; see the Perspective by **Masiello and Louchouart**) calculate that a large fraction of the charcoal produced by fires

is lost from the land through dissolution and transport to the oceans.

History Matters

Fishery-induced mortality is not the sole driver of fish population dynamics, and shifts in population abundance are also subject to ecological impacts. In a meta-analysis, **Neubauer et al.** (p. 347) showed how some fish-stock recoveries have been slower than theoretically predicted. They discovered that a species' exploitation history is also an important predictor of recovery. The data indicate that even extreme limitations in harvest pressure may not allow for the timely recovery of collapsed stocks.

Sliding Restriction

Helicase enzymes access the genetic information stored in double-helical DNA and RNA by opening the individual strands. Pseudo-helicases, including bacterial Type III restriction enzymes, use adenosine triphosphate (ATP) hydrolysis to communicate between two distant restriction sites on the same DNA and excise it only if the DNA is sensed as "foreign." **Schwarz et al.** (p. 353) show that the bacterial Type III restriction enzyme, EcoP15I, undergoes an ATP-dependent conformational switch that promotes sliding along the DNA to allow the enzyme to localize to its target.

Turn Off Youth

Our neurons regenerate better when we are young than when we are more mature. Studying the mechanosensory anterior ventral microtubule (AVM) neuron of the nematode *Caenorhabditis elegans*, **Zou et al.** (p. 372; see the Perspective by **Nix and Bastiani**) found that worms lacking microRNA machinery had unusually

robust axon regeneration and youthful-looking growth cones. Under- and overexpression of *let-7* microRNA confirmed its involvement in depressing axon regeneration.

Bats, Grids, and Oscillations

Nearly all animals move around in a three-dimensional (3D) world; however, very little is known about the neural circuitry underlying the representation of 3D space (see the Perspective by **Barry and Doeller**). Using whole-cell patch recordings in slices of entorhinal cortex, **Heys et al.** (p. 363) found that bat entorhinal stellate cells must generate grid patterns without theta-frequency oscillatory mechanisms. In another study, **Yartsev and Ulanovsky** (p. 367) used telemetry

to record activity from the hippocampus of bats while they were flying around. They found that active pyramidal cells—or place cells—in hippocampal area CA1 fired in positions, depending on where the animals were in the room.

Consciousness Arrives

Neurophysiological measures in human adults correspond to the transition between very brief, "unnoticeable," and slightly longer-lived visual stimuli that penetrate deeply enough to leave a conscious imprint that subjects report they can "see." **Kouider et al.** (p. 376) have performed parallel behavioral and neurophysiological studies in infants to identify a similar neural signal that appears to mark the development of visual consciousness.



Exhibits: November 17–19
Sessions: November 16–20
Resuscitation Science Symposium: November 16–17
Cardiovascular Nursing Symposium: November 19–20
Dallas, Texas



Abstract Submissions Are Now Open!

The American Heart Association's Scientific Sessions is the leading cardiovascular conference for basic, translational, clinical and population science. Lead the way to discovery in the fight against heart disease and stroke by submitting your best science today!

BENEFITS OF SUBMITTING YOUR SCIENCE:

GLOBAL REACH

OPPORTUNITY FOR MEDIA COVERAGE AND PUBLICATION

MEMBER BENEFITS

RECOGNITION FROM YOUR PEERS



Scan the barcode for more information about abstract submission.



Abstract Submission

April 17–June 5, 5 PM CDT | UTC -5 Hours



Late-Breaking Clinical Trial Application

June 3–24, 5 PM CDT | UTC -5 Hours



Late-Breaking Basic Science

July 29–Aug. 19, 5 PM CDT | UTC -5 Hours



Leading Discovery. Global Impact.

scientificsessions.org



Bruce Alberts is Editor-in-Chief of *Science*.

Prioritizing Science Education

THIS SPECIAL ISSUE OF *SCIENCE* EXPLORES “GRAND CHALLENGES IN SCIENCE EDUCATION,” A CRITICAL set of the problems and exciting opportunities now facing science education on a global level. The 20 Challenges, addressed by a team of education experts, range from “Enable students to build on their own enduring, science-related interests” to “Shift incentives to encourage education research on the real problems of practice as they exist in school settings.” Here I propose three additional Grand Challenges. These focus on harnessing the wisdom of teachers, helping the business community promote new directions in precollege science education, and—last but not least—catalyzing major changes in the way we teach college-level science.

From my many close contacts with outstanding U.S. teachers, I have come to deeply appreciate their wisdom. They uniquely understand today’s 5- to 18-year-old students and have many valuable suggestions for improving education systems. I am also painfully aware of the many past failures that have been caused by not giving the best teachers a strong voice in the public policies that profoundly affect their profession. In the 1980s, the Japanese taught the world that building a better automobile requires listening to workers on the assembly line. More generally, experience shows that actively soliciting advice from those most intimately involved is essential for wise decision-making at higher levels. Regrettably, education is one of the few parts of U.S. society that fails to exploit this fact. Hence, my initial Grand Challenge: “Build education systems that incorporate the advice of outstanding full-time classroom teachers when formulating education policy.” A start has been made,* but much more remains to be done (see the Perspective by B. Berry on p. 309).

To be competitive in the global economy, businesses need to be able to hire workers who can “think for a living.” More specifically, studies reveal that the private sector seeks employees who can apply a capacity for abstract, conceptual thinking to “complex real-world problems—including problems that involve the use of scientific and technical knowledge—that are nonstandard, full of ambiguities, and have more than one right answer.” These employees must also have “the capacity to function effectively in an environment in which communication skills are vital—in work groups.”† Achieving the revolution in U.S. science education that is called for in the *Next Generation Science Standards* released last week‡ would go a long way toward creating the type of high-school graduates that the private sector needs (see the Perspective by R. Stephens and M. Richey on p. 313). Business leadership in the United States often fails to advocate for wise education policies, despite its potential for influence. Hence, my second Grand Challenge: “Harness the influence of business organizations to strongly support the revolution in science education specified in the *Next Generation Science Standards*.”

Several years ago on this page, I pointed out that, “Rather than learning how to think scientifically, students are generally being told about science and asked to remember facts. This disturbing situation must be corrected if science education is to have any hope of taking its proper place as an essential part of the education of students everywhere. Scientists may tend to blame others for the problem, but—strange as it may seem—we have done more than anyone else to create it.”§ College science courses are taught by scientists, and they define “science education,” modeling for teachers and adults what should be done at lower levels. Most college faculty have not yet faced up to the urgent need to improve on the standard one-size-fits-all lecture format (see News story by J. Mervis on p. 292). Thus, my final Grand Challenge: “Incorporate active science inquiry into all introductory college science classes.”

The aim is nothing less than a more rational world.

— Bruce Alberts

10.1126/science.1239041



*<http://sites.nationalacademies.org/DBASSE/TAC>; www.ccst.us/ccstinfo/caltac.php. †R. Marshall, M. Tucker, *Thinking for a Living: Education and the Wealth Of Nations* (Basic Books, New York, 1993). ‡www.nextgenscience.org/ next-generation-science-standards. §B. Alberts, *Science* **323**,437 (2009).



ECOLOGY

Poor Replacements

The loss of large herbivore species can have very noticeable ecological impacts. Replacement of lost species with similar species expected to perform the ecological roles of those lost may temper some of these changes. Caution is needed, however: History is full of biological introductions that have not turned out as planned. Hunter *et al.* tested the effectiveness of ecological equivalency in the Galapagos, where extinctions of giant tortoises have had significant ecological impacts. Sterilized tortoises with two distinct shell morphologies, domed and saddleback, were introduced to Pinta Island. The now-extinct native species had saddleback-shaped shells and were dispersers of prickly pear cactus. Introduced saddleback tortoises also selected prickly pear-rich habitats and thus have the potential to fill the same ecological role. Tortoises with dome-shaped shells, on the other hand, migrated to high elevations and showed no inclination toward prickly pear habitat. This was somewhat surprising, because despite differences in shell shape, domed-shell tortoises are more closely related to the extinct saddleback tortoises. Controlled experimentation, therefore, may be essential when considering ecological replacement strategies. — SNV

Conserv. Biol. 10.1111/cobi.12038 (2013).

BIOCHEMISTRY

Transport by the Numbers

Twelve is a useful number when it comes to protein design. Why? Because divisibility by 2 and by 3 means that subunits or intraprotein domains can be assembled combinatorially while structural homologies insure stability. Madej *et al.* document a striking example of this flexibility in design by comparing the tertiary structures of two members of the major facilitator superfamily (MFS) of secondary transporters. MFS transporters carry small molecules across the

cell membrane, often in tandem with an ion; the movement of the small-molecule substrate, such as lactose or fucose, is driven by the electrochemical gradient of the ion, which is in turn established by active transporters. Both sugar permeases LacY and FucP consist of 12 transmembrane helices, and both of their secondary structures adhere to the canonical pattern of a quartet of three helix bundles. Biochemical analysis of mutant forms has identified the aromatic residues Trp¹⁵¹ and Phe³⁰⁸, respectively, as the platforms where the sugar substrates bind, but as is evident from their numbering, these

functionally homologous residues are found in entirely different locations in the primary structure. Madej *et al.* discovered that LacY and FucP are inverted permutations of each other, with bundles A through D in LacY corresponding to bundles D through A in FucP. — GJC

Proc. Natl. Acad. Sci. U.S.A. 110, 10.1073/pnas.1303538110 (2013).

GENOMICS

All-Seeing Mystery

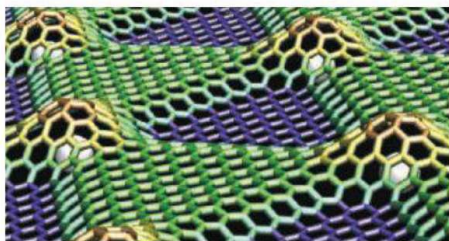
Nematode worms can infect a variety of tissues in humans; for example, apart from disconcertingly migrating across the eyeball on occasion, the African eyeworm *Loa loa* can cause neurological and heart pathologies. *L. loa* is a filarial worm, vectored by a biting fly and related to the worms that cause river blindness and elephantiasis. Unlike those species, *L. loa* does not possess the endosymbiont bacterium *Wolbachia*, which is the target of the antibiotics that are important treatments for these other parasites. Desjardins *et al.* have redressed a genome sequence gap and in a comparative study explored the nature of the apparently obligatory relationship between those filarial worms that have the endosymbiont and *L. loa*, which does not. It seems the eyeworm is very similar to other filariae to the extent that it (and they) possesses the metabolic pathways that previously were thought to be contributed by the endosymbiont. Contrarily, this new genome sequence is less revelatory than expected, instead restoring the symbiotic role played by *Wolbachia* in filarial nematodes to one of subtle mystery. — CA

Nat. Genet. 45, 10.1038/ng.2585 (2013).

MATERIALS SCIENCE

Implanted Atoms as Implements

Ion implantation can be used to remove a thin layer of a crystal. For example, in the fabrication of silicon-on-insulator chips, a layer of heteroatoms that can form a gas (e.g., hydrogen atoms) are implanted as a subsurface layer. Upon heating, the formation of the gas dislodges the thin film from the bulk crystal. Cun *et al.* examined such effects at the level of a single monolayer; in this case, a boron nitride (BN) overlayer grown on the (111) surface of rhodium that forms a “nanomesh” superstructure of wires and holes with a lattice constant of 3.2 nm. After implantation of low-energy argon ions, scanning tunneling microscopy revealed protrusions along the wires created by subsurface argon atoms trapped at two different sites. These structures were stable in air, but annealing to 900 K caused a “can-opener” effect; that is, the formation of well-defined 2-nm



holes in the perturbed overlayer along with the corresponding removed flakes of BN. Similar effects were seen with neon ions and for graphene layers on ruthenium surfaces. — PDS

Nano Lett. 10.1021/nl400449y (2013).

ECONOMICS

The Constraints of IP

Debates about intellectual property (IP) protections for genes remain unsettled. New analysis of landmark human genome sequencing efforts suggests that IP protections hindered use of some early findings in follow-on research and development (R&D). The publicly funded Human Genome Project (HGP) required that its results be immediately placed in the public domain. The private firm Celera, which published its draft genome at the same time as the HGP draft in 2001, maintained IP rights on its sequenced genes not yet sequenced by the HGP, allowing it to negotiate licensing agreements to sell data until those genes were made public by subsequent HGP resequencing, completed in 2003. Incorporating a range of counterfactuals and controls, Williams shows that scientific publications and the development of diagnostic tests based on genes covered by Celera's IP were reduced by 20 to 30%. Comparing Celera genes made public by the HGP in 2002 versus 2003, a gap remains in the overall stock of knowledge (measured by genes having a known phenotype), suggesting that even brief periods of IP can have persistent impacts on subsequent R&D. — BW

J. Polit. Econ. 121, 1 (2013).

APPLIED PHYSICS

Falling into Traps

Semiconducting nanocrystals, or quantum dots, are promising materials for use in laser, light-emitting diode (LED), and solar cell applications, partly because of the tunability of their photophysical properties with size. One important size-dependent property in this context is the rate of Auger recombination, a process whereby one or more excited carriers relax through energy transfer to another carrier. Cohn *et al.* report that, in zinc oxide nanocrystals negatively photodoped under anaerobic conditions, a distinct sort of

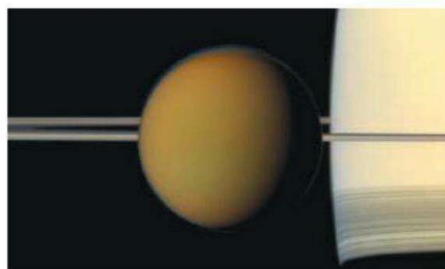
trap-assisted Auger recombination occurs at a rate that varies as the inverse square of the particle radius. Transient absorption and photoluminescence spectroscopy reveal the decay rates associated with relaxation of a conduction-band electron to a deep localized hole, through concomitant energy transfer to an extra conduction-band electron. Although the radial rate dependence is shallower than the inverse cube scaling of more widely studied biexciton recombination processes, the authors note that surface traps are common enough for the process to play a significant, and perhaps underappreciated, role in quantum dot photophysics. — JSY

Nano Lett. 13, 1810 (2013).

PLANETARY SCIENCE

Simulating Titan's Atmosphere

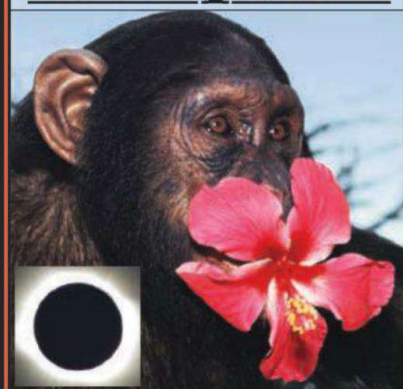
Saturn's largest moon, Titan, has a thick atmosphere dominated by nitrogen (N_2) and methane (CH_4); the photochemistry of these two gases in the upper part of the atmosphere, initiated by solar wind particles and solar ultraviolet radiation, produces organics, including polymeric compounds called tholins that are thought to give Titan its distinctive orange-yellow color. Laboratory work by Gudipati *et al.* shows that tholin formation can continue at low altitudes, down to 200 km from the surface and below, through longer-wavelength photochemistry in condensed organic aerosols. They synthesized dicyanoacetylene (C_4N_2), a compound that has been detected on Titan and can be used as a model system for other larger unsaturated condensing compounds, cooled



it to 100 K, a temperature relevant to Titan's lower atmosphere, and irradiated it with laser-produced light at 532, 355, and 266 nm. Solar light at wavelengths longer than 200 nm is expected to penetrate through Titan's atmosphere and make it to low altitudes without much attenuation. In the simulations, irradiation of C_4N_2 ice with light at wavelengths as long as 355 nm induced the formation of tholins. These organic compounds, which do not occur naturally on Earth, can thus be produced on Titan by different photochemical processes at different atmospheric altitudes. — MJC

Nat. Comm. 4, 1648 (2013).

AAAS Travels



Gorilla & Chimp Eclipse Safari

October 23–November 6, 2013

Total Solar Eclipse November 3

Safari in the "Pearl of Africa," Uganda, including the country's finest national parks, gorilla reserves & chimpanzee sanctuaries. \$5,495 + air + permits.

For a detailed brochure, please call (800) 252-4910

All prices are per person twin share + air

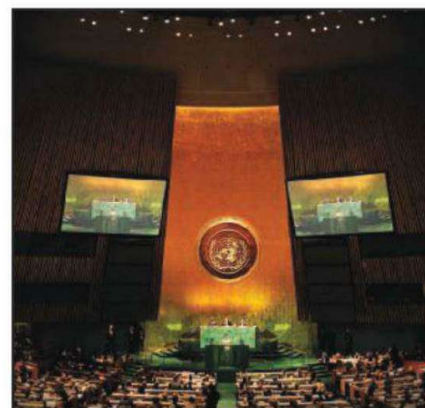


BETCHART EXPEDITIONS Inc.

17050 Montebello Rd, Cupertino, CA 95014

Email: AAASInfo@betchartexpeditions.com

www.betchartexpeditions.com

AAAS is here –
Science Funding, Climate
Regulation, Human Rights.

Around the world, governments turn to AAAS as an objective, multidisciplinary scientific authority to educate public officials and judicial figures on today's most pressing issues. And this is just one of the ways that AAAS is committed to advancing science to support a healthy and prosperous world. Join us. Together we can make a difference.

To learn more, visit
aaas.org/plusyou/policy



**1200 New York Avenue, NW
Washington, DC 20005**
Editorial: 202-326-6550, FAX 202-289-7562
News: 202-326-6591, FAX 202-371-9227
**Bateman House, 82-88 Hills Road
Cambridge, UK CB2 1LQ**
+44 (0) 1223 326500, FAX +44 (0) 1223 326501

SUBSCRIPTION SERVICES For change of address, missing issues, new orders and renewals, and payment questions: 866-434-AAAS (2227) or 202-326-6417, FAX 202-842-1065. Mailing addresses: AAAS, P.O. Box 96178, Washington, DC 20090-6178 or AAAS Member Services, 1200 New York Avenue, NW, Washington, DC 20005

INSTITUTIONAL SITE LICENSES please call 202-326-6755 for any questions or information

REPRINTS: Author Inquiries 800-635-7181
Commercial Inquiries 803-359-4578

PERMISSIONS 202-326-7074, FAX 202-682-0816

MEMBER BENEFITS AAAS Travels: Betchart Expeditions 800-252-4910; Apple Store www.store.apple.com/us/go/epstore/aaas; NASA Federal, 1-888-NASA-FCU (1-888-627-2328) or www.nasafcu.com; Cold Spring Harbor Laboratory Press Publications www.cshlpress.com/affiliates/aaas.htm; GEICO Auto Insurance www.geico.com/landingpage/go51.htm?logo=17624; Hertz 800-654-2200 CDP#3343457; Office Depot <http://bsd.officedepot.com/portalLogin.do>; Seabury & Smith Life Insurance 800-424-9883; Subaru VIP Program 202-326-6417; VIP Moving Services www.vipmayflower.com/domestic/index.html; Other Benefits: AAAS Member Services 202-326-6417 or www.aaasmember.org.

science_editors@aaas.org (for general editorial queries)
science_letters@aaas.org (for queries about letters)
science_reviews@aaas.org (for returning manuscript reviews)
science_bookrevs@aaas.org (for book review queries)

Published by the American Association for the Advancement of Science (AAAS), *Science* serves its readers as a forum for the presentation and discussion of important issues related to the advancement of science, including the presentation of minority or conflicting points of view, rather than by publishing only material on which a consensus has been reached. Accordingly, all articles published in *Science*—including editorials, news and comment, and book reviews—are signed and reflect the individual views of the authors and not official points of view adopted by AAAS or the institutions with which the authors are affiliated.

AAAS was founded in 1848 and incorporated in 1874. Its mission is to advance science, engineering, and innovation throughout the world for the benefit of all people. The goals of the association are to: enhance communication among scientists, engineers, and the public; promote and defend the integrity of science and its use; strengthen support for the science and technology enterprise; provide a voice for science on societal issues; promote the responsible use of science in public policy; strengthen and diversify the science and technology workforce; foster education in science and technology for everyone; increase public engagement with science and technology; and advance international cooperation in science.

INFORMATION FOR AUTHORS

See pages 716 and 717 of the 8 February 2013 issue or access.www.sciencemag.org/about/authors

SENIOR EDITORIAL BOARD

A. Paul Alivisatos, Lawrence Berkeley Nat'l Laboratory
Cort Barmann, The Rockefeller Univ.
Ernst Fehr, Univ. of Zurich
Erin O'Shea, Harvard Univ.
Michael S. Turner, University of Chicago

BOARD OF REVIEWING EDITORS

Adriano Aguzzi, Univ. Hospital Zürich
Takuzo Aida, Univ. of Tokyo
Leslie Aiello, Wenner-Gren Foundation
Sonia Altizer, Univ. of Georgia
Stefania Amigorena, Institut Curie
Angelika Amos, MIT
Kathryn Anderson, Memorial Sloan-Kettering Cancer Center
Siv G. E. Andersson, Uppsala Univ.
Peter Andolfatto, Princeton Univ.
Meinrat O. Andreae, Max Planck Inst., Mainz
Paola Ariotti, Harvard Univ.
Johan Auwerx, EPFL
David Awschalom, Univ. of California Santa Barbara
Ben Barres, Stanford Medical School
Jordi Bascompte, Estación Biológica de Doñana, CSIC
Facundo Batista, London Research Inst.
Ray H. Baughman, Univ. of Texas, Dallas
David Baum, Univ. of Wisconsin
Mark Bear, Massachusetts Inst. of Technology
Yasmine Belkaid, NIAID, NIH
Philip Benfey, Duke Univ.
Stephen J. Benkovic, Penn State Univ.
Christophe Bernard, Aix-Marseille Univ.
Gregory C. Berzosa, Stanford Univ.
Gabriele Bergers, Univ. of California, San Francisco
Peer Bork, EMBL
Bernard Bourdon, Ecole Normale Supérieure de Lyon
Chris Bowler, Ecole Normale Supérieure
Ian Boyd, Univ. of St. Andrews
Christian Büchel, Universitätsklinikum Hamburg-Eppendorf
Joseph A. Burns, Cornell Univ.
William P. Butz, Population Reference Bureau
György Buzsáki, Univ. of Oslo
Mats Carlsson, Univ. of Oslo
Mildred Cho, Stanford Univ.
David Clapham, Children's Hospital, Boston
David Clary, Univ. of Oxford
Jonathan D. Cohen, Princeton Univ.
Robert Cook-Deegan, Duke Univ.
James Collins, Boston Univ.
Alan Cowman, Walter & Eliza Hall Inst.
Robert H. Crabtree, Yale Univ.
Wolfgang Cramer, Mediterranean Inst. of Biodiversity and Ecology
Jeff L. Dangl, Univ. of North Carolina

Tom Daniel, Univ. of Washington
Frans de Waal, Emory Univ.
Stanislas Dehaene, Collège de France
Robert Desimone, MIT
Claude Desplan, New York Univ.
Ap Dijksterhuis, Radboud Univ. of Nijmegen
Dennis Discher, Univ. of Pennsylvania
Gerald W. Dorn II, Washington Univ. School of Medicine
Jennifer A. Doudna, Univ. of California, Berkeley
Julian Downward, Cancer Research UK
Bruce Dunn, Univ. of California, Los Angeles
Christopher Dye, WHO
David Ehrhardt, Carnegie Inst. of Washington
Tim Elston, Univ. of North Carolina at Chapel Hill
Gerhard Ertl, Fritz-Haber-Institut, Berlin
Barry Everitt, Univ. of Cambridge
Paul G. Falkowski, Rutgers Univ.
Ernst Fehr, Univ. of Zurich
Tom Fenchel, Univ. of Copenhagen
Michael Feuer, The George Washington Univ.
Alain Fischer, INSERM
Susan Fiske, Princeton Univ.
Anne C. Ferguson-Smith, Univ. of Cambridge
Peter Fratzl, Max Planck Inst.
Elaine Fuchs, Rockefeller Univ.
Wulfraam Gerstner, EPFL Lausanne
Andrew Gewirth, Univ. of Illinois
Karl-Heinz Glassmeier, TU Braunschweig
Elizabeth Grove, Univ. of Chicago
Kip Guy, St. Jude's Child's Research Hospital
Taekjip Ha, Univ. of Illinois at Urbana-Champaign
Christian Haass, Ludwig Maximilians Univ.
Steven Hahn, Fred Hutchinson Cancer Research Center
Gregory J. Hannam, Cold Spring Harbor Lab.
Martin Heimann, Max Planck Inst., Jena
Ysa Helariutta, Univ. of Finland
Isaac Held, NOAA
James A. Hendler, Rensselaer Polytechnic Inst.
Janet G. Hering, Swiss Fed. Inst. of Aquatic
Ray Hilborn, Univ. of Washington
Michael E. Himmel, National Renewable Energy Lab.
Kai-Uwe Hinrichs, Univ. of Bremen
Ken Hirose, Tokyo Inst. of Technology
David Hoadley, Univ. of Cambridge
David Holden, Imperial College
Lora Hooper, UT Southwestern Medical Ctr at Dallas
Jeffrey A. Hubbell, EPFL Lausanne
Thomas Hudson, Ontario Inst. for Cancer Research
Ray Huey, Univ. of Washington
Steven Jacobsen, UC Berkeley, Los Angeles
Kai Johnson, EPFL Lausanne
Peter Jonas, Universität Freiburg
Matt Kaeblerlein, Univ. of Washington

William Kaetzel Jr., Dana-Farber Cancer Inst.
Daniel Kamm, Harvard Univ.
Daniel Kammen, Univ. of California, Berkeley
Joel Kingsolver, Univ. of North Carolina at Chapel Hill
Robert Kingston, Harvard Medical School
Roberto Kolter, Harvard Medical School
Alberto R. Kornblith, Univ. of Buenos Aires
Leonid Kruglyak, Princeton Univ.
Thomas Kuhn, University of Vienna
Mitchell A. Lazar, Univ. of Pennsylvania
David Lazer, Harvard Univ.
Virginia Lee, Univ. of Pennsylvania
Ottoline Leyser, Cambridge Univ.
Marcia C. Lim, Univ. of California, Berkeley
Jianglu Liu, Michigan State Univ.
Luis Liz-Marzan, CIC bioMaGUNE
Jonathan Losos, Harvard Univ.
Ke Lu, Chinese Acad. of Sciences
Christian Lüscher, Univ. of Geneva
Laura Mackey, CRUK Beatson Inst. for Cancer Research
Anne Magurran, Univ. of St. Andrews
Oscar Marin, CSIC & Univ. Miguel Hernández
Charles Marshall, Univ. of California, Berkeley
Chris Marshall, Inst. of Cancer Research
Martin M. May, Baylor College of Medicine
C. Robertson McClung, Dartmouth College
Graham Medley, Univ. of Warwick
Yasushi Miyashita, Univ. of Tokyo
Richard Morris, Univ. of Edinburgh
Edward Muehleisen, Korean Univ. of Science and Technology
Sean Munro, MRC Lab. of Molecular Biology
Thomas Murray, The Hastings Center
Naoto Nagaosa, Univ. of Tokyo
James Nelson, Stanford Univ. School of Med.
Daniel Neumann, Univ. of California, Berkeley
Stuart Newman, New York Medical College
Timothy W. Nilsen, Case Western Reserve Univ.
Pär Nordlund, Karolinska Inst.
Helga Nowotny, European Research Advisory Board
Luke O'Neill, Trinity College, Dublin
Stuart Newman, New York Medical College
N. Phuan Ong, Princeton Univ.
Joe Orenstein, Univ. of California, Berkeley & Lawrence
Berkeley National Lab
Harry Orr, Univ. of Minnesota
Andrew Oswald, Univ. of Warwick
Steve Palumbi, Stanford Univ.
Jane Parker, Max-Planck Inst. of Plant Breeding Research
Donald R. Paul, Univ. of Texas at Austin
P. David Pearson, Univ. of California, Berkeley
John H. Petri, Memorial Sloan-Kettering Cancer Center
Simon Philpott, Univ. of Florida
Joshua Plotkin, Univ. of Pennsylvania
Philippe Poulin, CNRS

Colin Renfrew, Univ. of Cambridge
Trevor Robbins, Univ. of Cambridge
Jim Roberts, Fred Hutchinson Cancer Research Ctr.
Barbara A. Romanowicz, Univ. of California, Berkeley
Jens Rostrup-Nielsen, Haldor Topsøe
Mike Ryan, Univ. of Texas, Austin
Shimon Sakaguchi, Kyoto Univ.
Miquel Salmeron, Lawrence Berkeley National Lab
Jürgen Sandkühn, Medical Univ. of Vienna
Alexander Schier, Harvard Univ.
Randy Seeley, Univ. of Cincinnati
Vladimir Shalae, Purdue Univ.
Joseph Silk, Institut d'Astrophysique de Paris
Denis Simon, Arizona State Univ.
Alison Smith, John Innes Centre
Davor Solter, Inst. of Medical Biology, Singapore
Peter Sorger, Harvard Medical School
John Speakman, Univ. of Aberdeen
Allan C. Spradling, Carnegie Institution of Washington
Jonathan Sprent, Garvan Inst. of Medical Research
Paula Stephan, Georgia State Univ. and National Bureau of Economic Research
Elisbeth Stern, ETH Zürich
V. S. Subrahmanian, Univ. of Maryland
Ira Tabak, Columbia Univ.
Yoshiko Takahashi, Kyoto University
Sarah Teichmann, Cambridge Univ.
John Thomas, Duke Univ.
Herbert Virgin, Washington Univ.
Bert Vogelstein, Johns Hopkins Univ.
Cynthia Volkert, Univ. of Göttingen
Bruce D. Walker, Harvard Medical School
Douglas Wallace, Dalhousie Univ.
Ian Wamlsley, Univ. of Oxford
David A. Wardle, Swedish Univ. of Agric Sciences
David Waxman, Fudan Univ.
Jonathan Weissman, Univ. of California, San Francisco
Kathy Willson, Oxford Univ.
Ian A. Wilson, The Scripps Res. Inst.
Timothy D. Wilson, Univ. of Virginia
Rosemary Wyse, Johns Hopkins Univ.
Jan Zaanen, Leiden Univ.
Kenneth Zaret, Univ. of Penn. School of Medicine
Jonathan Zehr, Univ. of California, Santa Cruz
Maria Zuber, MIT

BOOK REVIEW BOARD

John Aldrich, Duke Univ.
David Bloom, Harvard Univ.
Angela Creager, Princeton Univ.
Richard Sweder, Univ. of Chicago
Ed Wasserman, DuPont
Lewis Wolpert, Univ. College London

EXECUTIVE EDITOR
Monica M. Bradford

MANAGING EDITOR, RESEARCH JOURNALS **Katrina L. Kelner**
DEPUTY EDITORS **Barbara R. Jasny, Andrew M. Sugden, Valda J. Vinson**

EDITORIAL SENIOR EDITORS/COMMENTARY Lisa D. Chong, Brad Wible; **SENIOR EDITORS** Gilbert J. Chin, Pamela J. Hines, Paula A. Kiberstis (Boston), Marc S. Lavine (Toronto), Beverly A. Purnell, L. Bryan Ray, Guy Riddihough, H. Jesse Smith, Phillip D. Szurmi (Tennessee), Jake S. Yeston, Laura M. Zahn (San Diego); **ASSOCIATE EDITORS** Melissa R. McCartney (Education Projects), Kristen L. Mueller, Jelena Stajic, Sacha Vignieri (Oregon), Nicholas S. Wigginton (Michigan); **BOOK REVIEW EDITOR** Sherman J. Suter; **ASSOCIATE LETTERS EDITOR** Jennifer Sills; **EDITORIAL MANAGER** Cara Tate; **SENIOR COPY EDITORS** Jeffrey E. Cook, Cynthia Howe, Harry Jach, Lauren Kmec, Barbara P. Ordway, Trista Wagoner; **COPY EDITOR** Chris Filiatreau; **SENIOR EDITORIAL COORDINATORS** Carolyn Kyle, Beverly Shields; **EDITORIAL COORDINATORS** Joi S. Granger, Anita Wynn; **PUBLICATIONS ASSISTANTS** Ramatoulaye Diop, Aneera Dobbins, Jeffrey Hearn, Lisa Johnson, Dona Mathieu, Le-Toya Mayne Flood, Shannon McMahon, Scott Miller, Jerry Richardson, Teresa R. Sakon, Brian White; **EDITORIAL ASSISTANT** Patricia M. Moore; **EXECUTIVE EDITORIAL ASSISTANT** Yolanda O'Bannon (San Francisco); **EXECUTIVE ASSISTANT** Alison Crawford; **ADMINISTRATIVE SUPPORT** Maryrose Madrid

EDITORIAL DIRECTOR, WEB AND NEW MEDIA Stewart Willis; **SENIOR WEB EDITOR** Sarah Crespi; **WEB EDITOR** Kerry Klein; **WEB DEVELOPMENT MANAGER** Martyn Green; **WEB DEVELOPER** Corinna Cohn
NEWS DEPUTY NEWS EDITORS Robert Coontz, Elizabeth Culotta, David Grimm (Online), Eliot Marshall, Jeffrey Mervis, Leslie Roberts, Richard Stone, John Travis; **CONTRIBUTING EDITOR** Polly Shulman; **NEWS WRITERS** Yudhijit Bhattacharjee, Adrian Cho, Jennifer Couzin-Frankel, Carolyn Gramling, Jocelyn Kaiser, Richard A. Kerr, David Malachuk, Elizabeth Pennisi, Robert F. Service (Pacific NW), Erik Stokstad, Emily Underwood; **WEB DEVELOPER** Daniel Berger; **SOCIAL MEDIA STRATEGIST** Meghna Sachdev; **INTERN** Lizzie Wade; **CONTRIBUTING CORRESPONDENTS** John Bohannon, John Cohen (San Diego, CA), Ann Gibbons, Sam Kean, Eli Kintisch, Andrew Lawler, Mitch Leslie, Charles C. Mann, Virginia Morell, Gary Taubes; **COPY EDITORS** Melissa Raimondi, Kara Estelle; **ADMINISTRATIVE SUPPORT** Scherraine Mack; **BUREAU** San Diego, CA: 760-942-3252, FAX 760-942-4979; Pacific Northwest: 503-963-1940

PRODUCTION DIRECTOR Wendy K. Shank; **ASSISTANT MANAGER** Rebecca Doshi; **SENIOR SPECIALISTS** Steve Forrester, Christopher Redwood, Anthony Rosen; **PREFLIGHT DIRECTOR** David M. Tompkins; **MANAGER** Marcus Spiegel; **SPECIALISTS** Jason Hillman, Tara Kelly
ART DIRECTOR Yael Fitzpatrick; **ASSOCIATE ART DIRECTOR** Laura Creveling; **SENIOR ILLUSTRATORS** Chris Bickel, Katharine Stuliff; **ILLUSTRATOR** Yana Hammond; **SENIOR ART ASSOCIATES** Holly Bishop, Preston Huey; **ART ASSOCIATES** Kay Engman, Garvin Grullion, Chrystal Smith; **PHOTO EDITOR** Leslie Blizard

SCIENCE INTERNATIONAL

EUROPE (science-int.co.uk) **EDITORIAL: INTERNATIONAL MANAGING EDITOR** Andrew M. Sugden; **SENIOR EDITOR/COMMENTARY** Julia Fahrenkamp-Uppenbrink; **SENIOR EDITORS** Caroline Ash, Stella M. Hurlley, Ian S. Osborne, Peter Stern; **ASSOCIATE EDITOR** Maria Cruz; **CONTRIBUTING EDITOR** Helen Pickersgill; **EDITORIAL SUPPORT** Rachel Roberts, Alice Whaley; **ADMINISTRATIVE SUPPORT** Janet Clements, Michelle Pegrum, John Wood; **NEWS: DEPUTY NEWS EDITOR**, U.K. Daniel Clerly; **CONTRIBUTING EDITOR**, Europe Martin Enserink; **CONTRIBUTING CORRESPONDENTS** Michael Balter (Paris), Kai Kupferschmidt (Berlin), Gretchen Vogel (Berlin)

ASIA Japan Office: Asca Corporation, Tomoko Furusawa, Rustic Bldg. 7F, 77 Tenjin-cho, Shinjuku-ku, Tokyo 162-0808, Japan; +81 3 6802 4616, FAX +81 3 6802 4615, inquiry@sciencemag.jp; **CONTRIBUTING EDITOR**, ASIA Mara Hvistendahl [China: mhvisten@aaas.org]; **CONTRIBUTING CORRESPONDENTS** Dennis Normile [Japan: +81 (0) 3 3391 0630, FAX +81 (0) 3 5936 3531; dnormile@gol.com]; Hao Xin [China: cindyhao@gmail.com]; Pallava Bagla [South Asia: +91 (0) 11 2271 2896; pbagla@vsnl.com]

EXECUTIVE PUBLISHER **Alan I. Leshner**
PUBLISHER **Beth Rosner**

FULFILLMENT SYSTEMS AND OPERATIONS (membership@aaas.org); **CUSTOMER SERVICE SUPERVISOR** Pat Butler; **SPECIALISTS** LaToya Casteel, Michelle Ofordire, April Marshall; **MANAGER, DATA ENTRY** Mickie Napoleoni; **DATA ENTRY SPECIALISTS** JJ Regan, Jaimee Wise, Fiona Giblin

BUSINESS OPERATIONS AND ADMINISTRATION DIRECTOR Deborah Rivera-Wienhold; **BUSINESS SYSTEMS AND FINANCIAL ANALYSIS DIRECTOR** Randy Yi; **SYSTEMS ANALYST** Nicole Mehmedovich; **MANAGER, BUSINESS ANALYSIS** Eric Knott; **MANAGER, BUSINESS OPERATIONS** Jessica Tierney; **BUSINESS ANALYSTS** Cory Lipman, Celeste Troxler; **FINANCIAL ANALYST** Jeremy Clay; **RIGHTS AND PERMISSIONS: ADMINISTRATOR** Emilie David; **ASSOCIATE** Elizabeth Sandler; **MARKETING DIRECTOR** Ian King; **MARKETING MANAGERS** Alison Chandler, Julianne Wielga, Justin Sawyers; **MARKETING ASSOCIATES** Mary Ellen Crowley, Elizabeth Sattler, Rebecca Riffkin; **SENIOR MARKETING EXECUTIVE** Jennifer Reeves; **DIRECTOR, SITE LICENSING** Tom Ryan; **DIRECTOR, CORPORATE RELATIONS** Eileen Bernadette Moran; **SENIOR PUBLISHER RELATIONS SPECIALIST** Kiki Forsythe; **PUBLISHER RELATIONS MANAGER** Catherine Holland; **PUBLISHER RELATIONS, EASTERN REGION** Keith Layson; **PUBLISHER RELATIONS, WESTERN REGION** Ryan Rexroth; **CUSTOMER RELATIONS MANAGER** Iquo Edim; **CUSTOMER RELATIONS ANALYSTS** Simon Chong, Lana Guz; **MARKETING MANAGER** Christina Schlecht; **MARKETING ASSOCIATES** Paulina Curto, Mitchell Edmund; **ELECTRONIC MEDIA DIRECTOR** Lizbeth Harman; **ASSISTANT MANAGER** Lisa Stanford; **PRODUCTION SPECIALISTS** Antoinette Hodal, Nichole Johnston, Lori Murphy, Kimberly Oster; **WEB AND NEW MEDIA: SENIOR PROJECT MANAGER** Trista Smith, **PROJECT LEADER** Luke Johnson **COMPUTER SPECIALISTS** Walter Jones, Kai Zhang, **WEB DEVELOPER** Chris Coleman; **PROGRAM DIRECTOR, AAAS MEMBER CENTRAL** Peggy Mihelich

DIRECTOR, GLOBAL COLLABORATION, CUSTOM PUBLICATIONS, ADVERTISING Bill Moran
EDITOR, CUSTOM PUBLISHING Sean Sanders; 202-326-6430

ASSISTANT EDITOR, CUSTOM PUBLISHING Tianna Hicklin 202-326-6463

ASSOCIATE DIRECTOR, COLLABORATION, CUSTOM PUBLICATIONS/CHINA/TAIWAN/KOREA/ SINGAPORE Ruolei Wu +86-1367-101-5294

PRODUCT (science_advertising@aaas.org); **MIDWEST** Rick Bongiovanni: 330-405-7080, FAX 330-405-7081; **EAST COAST/E. CANADA** Laurie Faraday: 508-747-9395, FAX 617-507-8189; **WEST COAST/W. CANADA** Lynne Stickrod: 415-931-9782, FAX 415-520-6940; **UK EUROPE/ASIA** Roger Gonçalves: TEL/ FAX +41 43 243 1358; **JAPAN, MAKIKO HARA**: +81 (0) 3 6802 4616, FAX +81 (0) 3 6802 4615; ads@sciencemag.jp; **CHINA/TAIWAN** Ruolei Wu: +86 1367 1015 294 rwu@aaas.org

WORLDWIDE ASSOCIATE DIRECTOR OF SCIENCE CAREERS Tracy Holmes: +44 (0) 1223 326525, FAX +44 (0) 1223 326532

CLASSIFIED (advertise@sciencerecruiting.org); **U.S./CANADA/SOUTH AMERICA** Tina Burks: 202-326-6577; **SALES ADMINISTRATOR** Marci Gallun; **EUROPE/ROW SALES** Lucy Nelson; **SALES ASSISTANT** Kelly Gager; **JAPAN** Yuri Kobayashi +81 (0)90-9110-1719; careers@sciencemag.jp; **CHINA/TAIWAN** Ruolei Wu: +86 1367 1015 294 rwu@aaas.org; **ADVERTISING SUPPORT MANAGER** Karen Foote: 202-326-6740; **ADVERTISING PRODUCTION OPERATIONS MANAGER** Deborah Tompkins; **SENIOR PRODUCTION SPECIALIST/GRAPHIC DESIGNER** Amy Hardcastle; **PRODUCTION SPECIALIST** Yuse Lajiminshup; **SENIOR TRAFFIC ASSOCIATE** Christine Hall; **SALES COORDINATOR** Shirley Young; **MARKETING MANAGER** Allison Pritchard; **MARKETING ASSOCIATE** Aimee Aponte

AAAS BOARD OF DIRECTORS RETIRING PRESIDENT, CHAIR William H. Press; **PRESIDENT** Phillip A. Sharp; **PRESIDENT-ELECT** Gerald R. Fink; **TREASURER** David Evans Shaw; **CHIEF EXECUTIVE OFFICER** Alan I. Leshner; **BOARD** Bonnie L. Bassler, May R. Berenbaum, Claire M. Fraser, Elizabeth Loftus, Stephen L. Mayo, Raymond Orbach, Sue V. Rosser, Inder M. Verma



ADVANCING SCIENCE. SERVING SOCIETY



Persistence

Kim Lewis, University Distinguished
Professor of Biology

Meet **Kim Lewis**, whose work on persister cells could refocus the direction of antibiotic drug development. He and his team have discovered that these bacterial cells survive antibiotic treatment by going dormant, and are using that discovery to explore novel therapeutics that could have a big impact on chronic infection treatment.

Learn how Lewis and our other faculty researchers are making the world healthier. Northeastern University: making tomorrow happen.

northeastern.edu/tomorrow

Northeastern University **Making Tomorrow Happen**



AROUND THE WORLD



Beijing 1

More H7N9 Infections Reported

A proliferation of newly reported infections has scientists and public health officials increasingly concerned about the pandemic potential of the H7N9 avian influenza strain in China. As *Science* went to press, the World Health Organization (WHO) said it had received reports of 63 cases, 14 of them fatal, in six Chinese provinces and municipalities. Scientists haven't yet pinpointed the source of the virus or the route by which people become infected. Although there is no evi-



More flu. A girl infected with the H7N9 bird flu strain is treated in a Beijing hospital on 14 April.

dence that it is spreading efficiently between people, which would herald a pandemic, "the situation is very worrisome," says influenza researcher Masato Tashiro of the National Institute of Infectious Diseases in Tokyo. One of the infections is in a 4-year-old boy who had no symptoms; there could be many more people with no or very mild symptoms, Tashiro says.

Last week, the China Center for Disease Control and Prevention in Beijing provided H7N9 samples to the other five laboratories in WHO's global network of collaborating centers for flu. These labs in turn can distribute the virus to other labs and companies that want to work with it. <http://scim.ag/H7N9cases>

Washington, D.C. 2

Supreme Court Weighs Human Gene Patenting

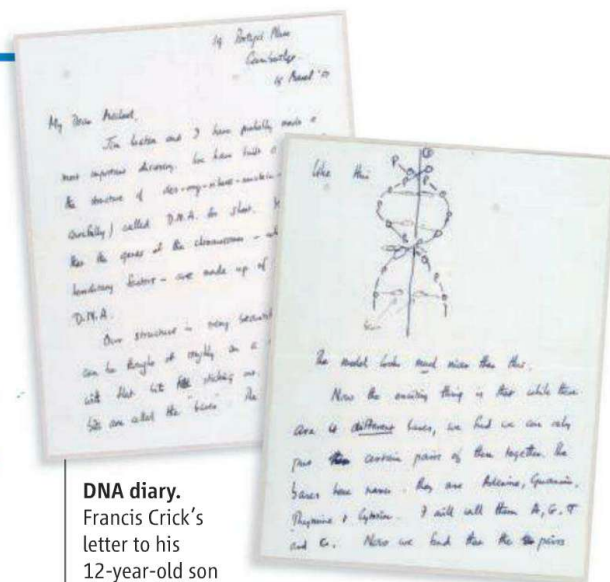
On 15 April, the U.S. Supreme Court heard arguments in a case that presents a simple but radical question: Can human genes be patented? Decades after the court gave a green light to the patenting of recombinant DNA, a group of clinicians and researchers, led by the American Civil Liberties Union and the Public Patent Foundation of New York City, wants to invalidate patents on human breast cancer genes (*BRCA1* and *BRCA2*) held by the diagnostic firm Myriad Genetics of Salt Lake City. The challengers say that DNA of this type is unpatentable because it is "a product of nature," not a human invention.

A lower federal court accepted this argument in 2010, tossing out Myriad's patents. An appeals court last year backed Myriad. The challengers went to the Supreme Court. There, the justices probed both sides with questions and metaphors—Justice Sonia Sotomayor compared gene patenting to baking cookies—seemingly in search of a compromise that would retain patents on artificial DNA but not on "natural" genes. A decision is due by the end of June. Coincidentally, Australia's highest court was also preparing to hear arguments on the validity of Myriad's patents this week.

New York, New York 3

Francis Crick Letter Sets Auction Record

"Jim Watson and I have probably made a most important discovery," begins a 1953 letter written by Francis Crick to his 12-year-old son. On 10 April, the 7-page letter, which describes the biologists'



DNA diary.

Francis Crick's letter to his 12-year-old son sold for a record \$5.3 million.

unraveling of the structure of DNA, was sold to an unidentified buyer for \$5.3 million at Christie's, setting a record price for a letter.

The next day, 11 other items from Crick's estate were auctioned off by Heritage Auctions, including the Nobel Prize medal that Crick won in 1962 (along with Watson and Maurice Wilkins) for the discovery of DNA's structure. The CEO of a regenerative medicine technology company bought the medal for a comparatively modest \$2.3 million. Half of the proceeds of the Heritage Auctions sales will go to the Salk Institute for Biological Studies in San Diego, California, where Crick worked from 1976 to 2004. Another 20% of the proceeds will go to the London-based Francis Crick Institute, which is to open in 2015.

Washington, D.C. 4

Flawed Informed Consent Claimed in Premie Study

Researchers from 23 universities involved in a study of 1316 extremely premature infants failed to "disclose adequately" that the infants could become blind or die depending on the concentration of oxygen that they received, concluded a 7 March letter from the U.S. Office for Human Research Protection (OHRP). The letter was brought to a reporter's attention last week by Public Citizen, a Washington, D.C.-based nonprofit group. The infants were in the SUPPORT trial, which was led by the University of Alabama, Birmingham, and ran from 2005 to 2009. SUPPORT's results, published in 2010 in



Rolling With the Budget

President Barack Obama's 2014 budget request to Congress demonstrates once again that, when it comes to science, "He gets it," says presidential science adviser John Holdren. Speaking last week at AAAS (which publishes *Science*), Holdren (at podium), offered an overview of the administration's proposed \$143 billion investment in research and development. He was joined by, from left: White House official Patricia Falcone, NASA Administrator Charles Bolden, National Institutes of Health Director Francis Collins, acting National Science Foundation Director Cora Marrett, and acting National Oceanic and Atmospheric Administrator Kathy Sullivan. The 2014 request is \$12 billion over the sequestration-depressed amount allocated this year and represents a 1.3% boost over 2012 levels. Civilian research would grow by 9%, while defense spending would shrink by 5%. For full budget coverage, see page 257.

The New England Journal of Medicine, concluded that infants receiving lower oxygen levels (blood saturation target of 85% to 89%) were more likely to die, but less likely to become blind, than babies receiving higher levels (91% to 95%). Although both levels were within the accepted standard of care, Public Citizen suspects that "many, if not most, parents would have declined to enroll" in SUPPORT had they fully understood the risks.

Bad Berleburg, Germany 5

Bison Back in Western Europe's Wild

European bison are back in the wild in Germany for the first time since the 18th century. Eight animals—one bull, five female adults, and two calves—were released on 11 April in a large private forest in the Rothaar Mountains, in North Rhine-Westphalia.

After many decades of extinction in the wild, European bison populations have recovered thanks to captive breeding and reintroduction programs. Of the 4000 in the world, about 3000 are roaming freely in Eastern European countries including Poland and Belarus.



But the German program is the first of its kind in Western Europe: a "historic moment," says Diana Pretzell, the World Wide Fund for Nature's manager for nature protection in Germany. The project benefits

from the experience of previous reintroduction efforts, Pretzell adds: In particular, scientists provided advice to minimize inbreeding in the new herd.

The reintroduction project was put forward by the forest's owner, logging businessman Prince Richard of Sayn-Wittgenstein-Berleburg. It has received a combined €1.58 million (about \$2.1 million) from Germany's federal agency for nature conservation and North Rhine-Westphalia's state government.

NEWSMAKERS

IVF Pioneer Dies

British physiologist **Robert Edwards** died last week in his sleep at age 87, nearly 35 years after he helped usher the first "test-tube" baby into the world. Edwards and gynecologist Patrick Steptoe developed the technique of in vitro fertilization (IVF), which has helped millions of couples around the world have children, and for which Edwards was awarded the Nobel Prize in physiology or medicine in 2010. (Steptoe died in 1988.)



Edwards's efforts began in the 1950s, when he studied how human eggs mature and under what conditions fertilization can succeed. He reached out to physician Steptoe to help bring these lab discoveries to couples battling infertility. The two transferred dozens of embryos before one developed into a healthy pregnancy: Louise Brown was born in 1978.

"Probably not since the invention of nuclear weapons has a scientific advance been received with such mixed feelings," wrote *The New York Times* in an editorial published 3 days later. While cutting-edge reproductive technologies still ignite debate, the ethical handwringing over IVF has long since settled down, and the treatment has become a mainstream part of fertility care.

Science LIVE

Join us on Thursday, 25 April, at 3 p.m. EDT for a live chat on the **Anthropocene**. Have humans altered the planet enough to earn our own epoch? When did it begin?
<http://scim.ag/science-live>

BY THE NUMBERS

\$3000 to \$5000 Cost to generate a human genome sequence in 2013, compared with \$1 billion when the Human Genome Project started in 1990 and \$10 million to \$50 million when it ended in 2003.

40 to 55 Percent of Tamiflu prescriptions that went unused during the 2009 to 2010 H1N1 swine flu pandemic, according to a study in *PLOS ONE* that estimated drug compliance rates based on sewage water.

\$13.6 billion Amount that Thermo Fisher Scientific of Waltham, Massachusetts, will pay for Carlsbad, California-based biotech equipment manufacturer Life Technologies.

FINDINGS

Hares Wear Winter White, Even When It's Warm

For hares, fashion is life or death. Blending in with the environment is how they avoid being eaten: In winter, their coats turn white; in summer they are a mottled brown. This wardrobe switch starts at about the same time each spring and fall, a new study shows—which means that as the climate warms, by midcentury hares could be more than a month out of sync with snow cover.

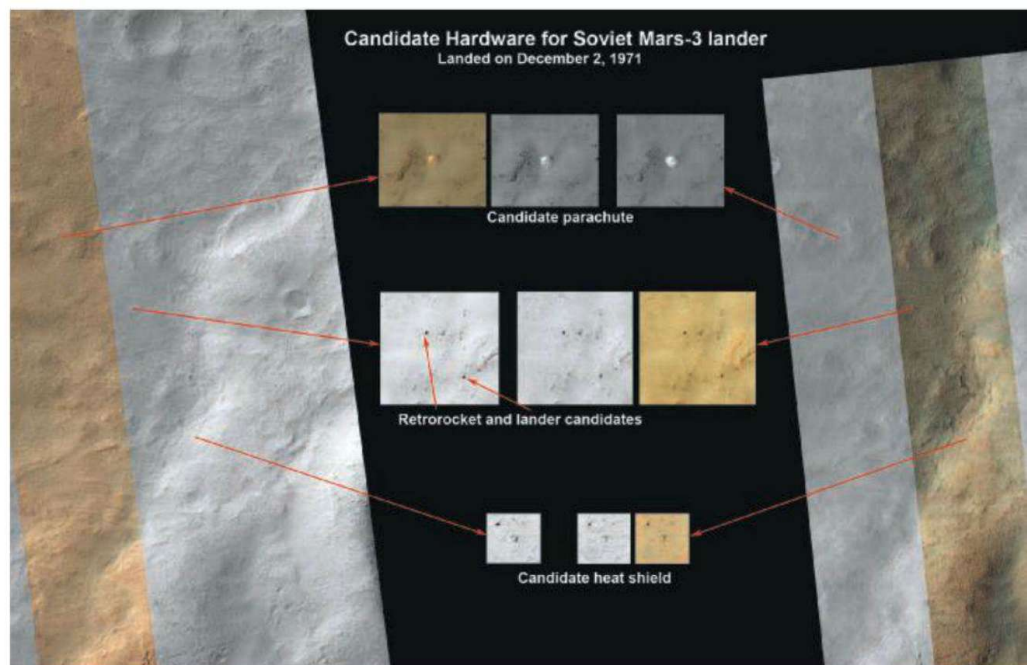
L. Scott Mills, a wildlife biologist at the University of Montana, Missoula, and his colleagues put radio tags on about 50 hares



Deadly mismatch. Climate change could put color-changing hares out of sync with snow cover.

during the winters of 2010, 2011, and 2012. Every week, the team monitored how well each hare matched its background.

Although one winter included 160 days of snow cover and another 190 days, the hares had trouble changing their coats to



Long-Lost Mars Lander Found?

About a week after the unmanned Soviet probe Mars 2 crashed into the planet in 1971, its twin, Mars 3, became the first spacecraft to manage a soft landing on Mars. Mars 3 looked around for about 15 seconds before its transmission mysteriously stopped. On 11 April, NASA announced that its Mars Reconnaissance Orbiter may have spotted the errant Mars 3 lander, along with detritus from its descent—its parachute, heat shield, and terminal retrorocket. The images, taken with the orbiter's High Resolution Imaging Science Experiment (HiRISE) camera, were actually captured about 5 years ago; an online community of Russian space enthusiasts pored over the images to find viable candidates for the expected hardware. The features appear to be a good match to the lander's components, HiRISE Principal Investigator Alfred McEwen said in a NASA press release, but further analysis "may help to confirm this interpretation."

match, Mills and his colleagues report online this week in the *Proceedings of the National Academy of Sciences*. The results "provide a really compelling visual effect of climate change," says Daniel Blumstein, a behavioral ecologist at the University of California, Los Angeles. <http://scim.ag/winterbun>

More Hints of Dark Matter?

Physicists working with a supersensitive particle detector deep underground have spotted three events that could be particles of dark matter bumping into atomic nuclei. However, that's too few events to say anything definite, caution the researchers, who work with the Super Cryogenic Dark Matter Search (SuperCDMS) experiment that sits 713 meters down in the idled Soudan mine in northern Minnesota. "We certainly

think it's interesting, but it doesn't rise to the level of discovery," says Blas Cabrera, a SuperCDMS team member from Stanford University in Palo Alto, California. The events could be "background" from ordinary particles, although the team estimates the chances of that at 0.2%.

The hint, reported at a meeting of the American Physical Society in Denver, has others excited. "I'm thrilled!" says Rafael Lang of Purdue University in West Lafayette, Indiana, who works on the competing XENON Dark Matter Project. He notes that yet another experiment called CoGeNT in Soudan has reported similar, but more ambiguous signals. "This is how discoveries happen," Lang says, "they creep in and at some point you can't explain it away anymore." Look for results from XENON and other experiments within a year or two.

CREDITS (TOP TO BOTTOM): NASA/JPL-CALTECH/UNIV. OF ARIZONA; L. SCOTT MILLS ET AL., PNAS EARLY EDITION (2013)

2014 U.S. BUDGET

Request Would Let Science Rebound From Sequester

He's done it again. And for most U.S. scientists, that's good news.

President Barack Obama last week submitted a \$3.8 trillion budget request to Congress for 2014 that, if enacted, would boost the research budgets of nearly every federal agency. His continued support for science stands out in an otherwise flat budget that aims to shrink the federal deficit by clamping down on entitlement programs and raising money by revising the tax code.

The president's spending blueprint for 2014 should lift the spirits of a community that, along with all other sectors of the economy, has endured a bumpy political ride for the past year. The president's \$143 billion request for research and development more than erases a nearly \$10 billion dip from 2012 to 2013 caused by sequestration—the \$85 billion, across-the-board cut in discretionary spending that went into effect in March. A few weeks later, Congress extended a budget freeze for most agencies through 30 September, the end of the 2013 fiscal year (*Science*, 29 March, p. 1511).

Like its predecessors, the 2014 request favors three agencies that provide significant support in the physical and computing sci-

ences and engineering. It seeks a 10.8% boost over this year's sequestration-reduced budget for the National Science Foundation (NSF), a 10.5% hike for the Office of Science within the Department of Energy, and a 19.8% jump for the core labs at the National Institute of Standards and Technology. This year that trio is joined by the U.S. Geological Survey, for which the administration has requested a 15.2% increase.

Obama's latest budget (see boxes below for details) even provides a lifeline for the beleaguered biomedical research community by offering a 6.8% bump over 2013 for the National Institutes of Health. Only a handful of science agencies, notably the Centers for Disease Control and Prevention and the Environmental Protection Agency, took hits this year. But a proposed reorganization of federal programs to improve science, technology, engineering, and mathematics (STEM) education at all levels has roiled parts of the scientific community (see p. 258).

Keeping track of winners and losers is a bit harder this year because the administration has chosen to compare its 2014 request with 2012 rather than 2013 spending levels. White House officials say they didn't have

time to incorporate the 2013 totals. The odd scorekeeping masks the impact of sequestration, which was the penalty that the White House and Congress imposed on themselves as part of a 2011 budget agreement if the two sides were unable to find another way to shrink the budget deficit over the next decade.

Obama has ridiculed sequestration as bad public policy, and his new budget would replace the across-the-board cuts in 2014 with a combination of more targeted spending cuts and revenue increases. So perhaps the White House just doesn't want to talk about the topic. "The effect of the sequester is already being felt in many domains," said John Holdren, the president's science adviser, in a presentation last week of the 2014 science budget. "So the sooner we get rid of it, the better."

Although all agencies were dinged by the sequester—civilian agencies took a 5% cut, and national security programs were trimmed by 7.4%—the final spending bill for 2013 softened the blow for some, in particular, NSF. Federal agencies are putting the finishing touches on operating plans describing how this year's cuts apply to every program.

Those plans involve real money, and the allocation will affect every researcher who receives federal dollars. In contrast, the president's budget is a wish list, and its arrival marks just the beginning of what will certainly be another protracted debate over federal spending. Even so, it's being greeted warmly by a community eager to bury the unpleasant memories of 2013.

—JEFFREY MERVIS

Applause for small favors



The president's fiscal year 2014 budget offers the National Institutes of Health (NIH) a modest 1.5% increase over 2012 levels. But the request would also erase the 5% cut that the agency received this year, to \$29.3 billion, because of the mandatory across-the-board cuts known as sequestration. "Everything's relative," said NIH Director Francis Collins after a press briefing. "Considering what we've been going through in FY '13, what's being proposed here is really gratifying." NIH would fund 10,269 new grants, an increase of 351 over 2012. The \$471 million in new funding includes \$40 million for NIH's portion of the BRAIN Initiative unveiled by the president last month. "It's a little bit of good news, but I think it's still very worrisome as to where this is all headed," said David Moore of the Association of American Medical Colleges.

Up close with an asteroid



Find a 454-tonne asteroid. Grab it. Drag it close to the moon. That's the idea behind an audacious initiative spelled out in NASA's 2014 budget proposal. The agency wants to spend \$105 million on the plan across the exploration, aeronautics, and science directorates. That figure includes doubling its \$20 million program for asteroid detection. Once it has found the right-size asteroid, NASA intends by 2019 to send a spacecraft to capture it in a giant bag and pull it into an orbit around the moon. Then, astronauts would visit the asteroid aboard the Orion space capsule, currently under development. "This mission raises the bar for human exploration and discovery," says NASA Administrator Charles Bolden.

Will red rover come over?



A year ago, U.S. planetary scientists were dismayed when NASA announced that it was backing out of a European-led plan to explore Mars. But now it has requested money to send a new rover to Mars in 2020 that will ultimately return samples to Earth, a top priority in the most recent planetary science decadal survey. The 2014 budget proposal does not specify how much the new mission will cost or what the agency wants to spend next year. But budget documents explain that the new rover would be based on the design of Curiosity—the rover currently exploring Mars—which NASA says "will ensure mission costs and risks are as low as possible." John Mustard, a planetary scientist at Brown University, says "it is great to see the semblance of a start for a rover to be launched in 2020."

2014 U.S. BUDGET

Wild Cards Remain After Proposed Reshuffle of STEM Education

Most people agree that the U.S. government could do a better job of managing the \$3 billion being spent by a dozen federal agencies on science education, public understanding of science, and the training of future scientists and engineers. But few are lining up behind a White House plan to accomplish that goal.

In a move that surprised science educators, legislators, and even many federal education officials, President Barack Obama included a radical realignment of STEM (science, technology, engineering, and mathematics) education programs in the 2014 budget request that he submitted last week to Congress (see p. 257). He proposed eliminating dozens of programs at agencies—notably at NASA, the National Institutes of Health (NIH), and the National Oceanic and Atmospheric Administration (NOAA)—in which STEM education is an add-on to their primary missions. At the same time, his budget designates three agencies to shoulder the load in four priority areas—the Department of Education in precollege science and math education, the National Science Foundation in both undergraduate and graduate STEM training, and the Smithsonian Institution in STEM activities outside the regular classroom.

The realignment will “substantially decrease the fragmentation of STEM programs,” says John Holdren, the president’s science adviser and director of the White

House Office of Science and Technology Policy (OSTP). The General Accountability Office, a congressional watchdog agency, has found that 83% of STEM education pro-



grams “overlapped to some degree ... [by] offer[ing] similar services to similar target groups ... to achieve similar objectives,” and many Republican legislators assert that federal STEM education programs are poorly managed and even redundant.

Under the proposed reshuffling, some 78 programs with an overall budget of \$176 million would be eliminated. But offi-

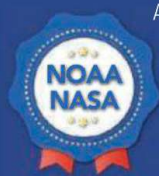
cials at the White House Office of Management and Budget (OMB), which developed the proposal, declined to explain what criteria were used in swinging the ax. And those cuts won’t result in overall savings. Instead, the president’s budget calls for a \$195 million jump in funding on STEM education over 2012, with the Education Department receiving an additional \$285 million, NSF enjoying an \$89 million boost, and the Smithsonian getting \$25 million to join the roster of federal STEM education agencies.

Many science educators say that the proposed cuts would scrap effective programs just as the country needs to be doing more. “The SEPA [Science Education Partnership Award] program is the face of NIH to the country,” says a grantee on one of the programs facing the chopping block. “It’s a vehicle for telling the public how NIH is translating science into practice,” says the grantee, who requested anonymity.

Conservatives are unhappy with the proposal’s bottom line. “The president’s proposal includes a reduction or reorganization of 114 of the 226 federal STEM programs, but then requests almost 7% in additional funding for the remaining 112 programs,” says Representative Larry Bucshon (R-IN), chair of the research subcommittee of the House of Representatives science committee. “I have yet to see sufficient justification for this additional funding.”

Even ardent backers of STEM education are wary of what the president has proposed. “The consolidation is not straightforward and not without possible consequences,” said a spokesperson for Representative Eddie

Here’s looking at you, kid



A 15-year satellite soap opera may have reached its final scene. The administration has requested \$33.6 million to finish refurbishing and launch the Deep Space Climate Observatory (DSCOVR), a satellite proposed by then-Vice President Al Gore in 1998. It was supposed to launch in 2001 and beam back climate data and pictures of the Earth from a point 1.5 million kilometers away (*Science*, 20 March 1998, p. 1845). But critics saw the satellite, then called Triana, as a political stunt, and the George W. Bush administration parked it in a warehouse. The Obama administration revived the mission in 2010 and now wants to give \$9.9 million to NASA and \$23.7 million to the National Oceanic and Atmospheric Administration to get DSCOVR launched in November 2014.

Cosmic movie ready to roll



Astronomers will be thrilled that the National Science Foundation (NSF) has requested \$27.5 million to begin construction of the Large Synoptic Survey Telescope (LSST), the community’s top choice for ground-based facilities in its 2010 decadal survey. Sitting atop Cerro Pachón in Chile, the 8.4-meter scope would use a 3 gigapixel camera to map an entire hemisphere of the sky once every 4 days. The result will be an unprecedented 3D movie of the cosmos and the mapping of some 10 billion galaxies. DOE has proposed spending \$22 million from its high-energy physics program to continue building the camera. (Overall, the program would get a boost of 5.7%.) Organizers hope to complete work on the telescope in 2021, using \$466 million from NSF and \$173 million from DOE.

A compromise for fusion



The 10.6% boost for the U.S. Department of Energy’s (DOE’s) Office of Science would allow the agency to pay the country’s share of the international fusion experiment ITER, under construction in France, without gutting fusion research at home. Funding for fusion research would climb more than 20% to \$458 million, of which \$225 million would go to building parts for ITER. But DOE would limit annual ITER contributions to that amount for the duration of construction, says Edmund Synakowski, DOE’s associate director for fusion energy sciences. That move would prevent ITER from consuming the entire fusion budget, although it would increase the total amount the United States will pay for its share of ITER. DOE still plans to shut down a tokamak at the Massachusetts Institute of Technology.

CREDIT: CARLA SCHAEFER/AAAS

Bernice Johnson (D-TX), the top Democrat on the science committee. “Agencies have unique mission and workforce needs and expertise that can’t easily be replicated by other agencies. Until she sees the details, she can’t judge what makes sense.”

Johnson isn’t the only one who’s confused. Federal officials, who learned about the plan during negotiations with OMB on their 2014 budget requests, say they still have a lot of questions. “The proposal has just been released and we are awaiting details,” says Lawrence Tabak, principal deputy director at NIH. “We’re in a funding pause for new applications.” Grantees, however, say they were told last week in a conference call that NIH intends to shutter its Office of Science Education and shift responsibility for STEM education activities to individual institutes and centers.

The announcement troubles Carl Wieman, who until last June oversaw federal STEM education programs as assistant OSTP director for science (see p. 292). An interagency committee has spent 4 years working on a strategic plan to consolidate and coordinate existing STEM education programs and beef up assessment, he notes. Its vision, long overdue, is now expected to be unveiled next month. By circumventing that process, Wieman says, OMB “is throwing a stick of dynamite into the room. It screws up the whole process.”

Steve Robinson of the White House Domestic Policy Council disagrees. He says the realignment is actually “one step in

achieving those goals, by realigning efforts and redirecting resources around more clearly defined priorities and enabling rigorous evaluation.”

While the dust settles, the three lead agencies are hoping that Congress will bless



Some lose. NASA’s Charles Bolden and NIH’s Francis Collins would see education programs shrink.

their plans to expand existing programs. The Smithsonian wants to apply its windfall to its newly named Center for Learning and Digital Access, which offers easy access to Smithsonian documents, photos, podcasts, videos, and other materials aligned with state standards in STEM fields and will eventually include materials from other agencies. The Department of Education wants to launch a \$150 million competitive grants program to help local school districts prepare high school graduates for STEM majors and careers and plans to create a STEM office that would work with other federal science agencies.

NSF hopes to grow its prestigious graduate research fellowships program from 2000 new fellows a year to 2700. Some of the fellows would have a chance to develop “special knowledge or skills” in collaboration with mission agencies, says Joan Ferrini-Mundy, the head of NSF’s education directorate. “The big picture is that some programs from other agencies are winding down,” she says. “And as we ramp up our undergrad and graduate programs, we’ll be talking to those agencies about access to their assets and facilities.”

The White House has not spelled out how such transfers would occur. Although funding for NASA’s STEM education activities would drop from \$170 million to \$109 million under the realignment, for example, NASA will continue to offer internships for undergraduates as well as funding students at colleges and universities with large minority populations. Likewise, NSF would retain its informal science education and K–12 programs.

Outside observers think it is unlikely that Congress will agree to anything as radical as what the White House has proposed. And mission agencies aren’t eager to give up what they have been doing. “We have the capability to play in all four priority areas,” notes Leland Melvin, head of NASA’s Office of Education, “as well as world-renowned scientists and facilities that nobody else has. NASA will continue in 2013 to do what we’ve been doing, and we’ll see what happens after Congress has a chance to weigh in. It’s a process.”

—JEFFREY MERVIS

Biodefense on the farm



The Obama administration has decided to move ahead with a controversial agro-defense laboratory planned for Manhattan, Kansas. It has requested \$714 million to enable the Department of Homeland Security (DHS) to build the \$1.2 billion National Bio and Agro-Defense Facility (NBAF), a high-security laboratory that would study diseases that threaten humans and livestock. NBAF is supposed to replace the Plum Island Animal Disease Center in New York. Just last year, the White House omitted NBAF funds in light of safety and budget concerns, as well as opposition from members of Congress. But some supportive outside reviews appear to have helped revive NBAF for 2014. Fierce opposition in Congress remains, however, with Representative Tim Bishop (D-NY) vowing to kill what he calls a “billion-dollar boondoggle.”

Giving a lift to science



The president’s 2014 request confirms that the U.S. Geological Survey (USGS) has moved up in the scientific pecking order. After years of 1% and 2% proposed bumps, in 2013 the administration asked for a 3% boost. That increase didn’t happen, as the agency suffered the same 5% cut from sequestration as its peers. But this year the president came back with a plan for a 15.2% hike over current levels, to \$1.17 billion. Marcia McNutt, who stepped down in February as USGS director and who in June becomes editor-in-chief of *Science*, says the recent budget jumps reflect the survey’s shift in status from a subsidiary of the Department of the Interior to a full-fledged science agency advancing the administration’s science priorities. One of those priorities is climate change research, which is slated for a 22% increase to \$72 million.

Chewing over a big request



The White House has doubled down on its support for competitively awarded, peer-reviewed funding for research. Last year, it asked for a 23% boost to the Agriculture and Food Research Initiative (AFRI) while holding level its requests for other research at the U.S. Department of Agriculture. That request was rejected, but this year it is seeking a 44% boost over current levels, to \$383 million. Karl Glasener, who directs science policy for the American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, says a 2012 report by the President’s Council of Advisors on Science and Technology helped make the case. Congressional approval is another matter, but Glasener is optimistic: “Congress too seems increasingly sold on competitive funding mechanism in general and for AFRI specifically.”

**eppendorf
& Science**

**PRIZE FOR
NEURO
BIOLOGY**



2012 Winner
Dr. Marlene R. Cohen
Assistant Professor
University of Pittsburgh

Call for Entries

**Application Deadline
June 15, 2013**

Eppendorf & Science Prize for Neurobiology

The annual Eppendorf & Science Prize for Neurobiology, an international award, honors young scientists for their outstanding contributions to neurobiological research. The winner and finalists are selected by a committee of independent scientists, chaired by Science's Senior Editor, Dr. Peter Stern. To be eligible, you must be 35 years of age or younger.

You could be next to win this prize and to receive

- > Prize money of US\$25,000
- > Publication of your work in Science
- > Full support to attend the Prize Ceremony held in conjunction with the Annual Meeting of the Society for Neuroscience in the USA
- > An invitation to visit Eppendorf in Hamburg, Germany

It's easy to apply!

Learn more at: www.eppendorf.com/prize

eppendorf





ARCHAEOLOGY

Archaeologists Say the 'Anthropocene' Is Here—But It Began Long Ago

HONOLULU—A vocal group of geologists and other scientists are pushing to define a new geological epoch, marked by climatic and environmental change caused by humans. They've got a catchy name—the Anthropocene or "Age of Man"—plus an eponymous journal starting up this month and an international working group considering whether to make the designation official (*Science*, 7 October 2011, p. 32). At a session at the Society for American Archaeology* meetings here, archaeologists argued that it's high time for their field, which studies humans and their activities over geological time, to have a greater voice in the debate.

Most all the speakers at the standing-room-only session agreed that human impacts on the Earth are dramatic enough to merit a new epoch name—and that drawing attention to our changed relationship with the planet is a good idea. But all felt that such an epoch should start thousands of years ago. Rather than focusing on a relatively sudden planet-wide change—a line in a sedimentary deposit where one could plant a "golden spike" as is traditionally done to mark an epoch—the archaeologists outlined an unbroken chain of human influence stretching far back into prehistory. "Humans have been modifying ecosystems over a long period of time," said archaeologist Bruce Smith of the Smithsonian Institution's National Museum of Natural History (NMNH) in Washington, D.C.

Such a view of the Anthropocene contrasts with that of most geologists and ecologists behind the movement, many of whom have

sought a clear time marker for the "golden spike." The most popular suggestion for a time point to end the recognized Holocene epoch, which began 11,700 years ago after the end of the last ice age, is the beginning of the Industrial Revolution about 1750 C.E., when manufacturing began to take hold and human-caused global warming is thought to have begun. Only then, proponents argue, did humans begin to change the chemistry of the atmosphere and oceans worldwide, alter the course of rivers, and transform the landscape, among other impacts. Others have suggested fallout from nuclear bombs as a distinctive Anthropocene signature.

But although the 23-member Working Group on the Anthropocene has no archaeologists or anthropologists, those fields also have bountiful data on major human impacts on the planet, said session chair Todd Braje, an archaeologist at Humboldt State University in Arcata, California. Those data show that humans began making global and large-scale transformations of the Earth long before the 18th century, panel members said.

Jon Erlandson, an archaeologist at the University of Oregon in Eugene, noted that people began transforming continents about 60,000 years ago, when our ancestors began to spread out from their ancestral home in Africa. That's when large-scale hunting, burning vegetation, and cutting down forests, etc., began, and "set the stage for human domination of the earth," Erlandson said.

Shortly after humans arrived in many regions, including most islands and several continents, many large mammals went extinct. Because the climate changed natu-

Heavy footprint. Humans have been transforming landscapes, such as these ancient Philippine rice fields, for thousands of years.

rally just as the humans arrived, it's still not clear whether hunting, climate, or both killed off the megafauna, Braje says. He noted 2011 work led by Eske Willerslev of the University of Copenhagen, which concluded that climate alone best explains some extinctions, but that both climate and human hunting drove Eurasian steppe bison and wild horses extinct, for example. Thus, human-caused extinctions go back thousands of years.

Instead of focusing on the Anthropocene's measurable effects, which accumulated over time, some archaeologists argued that perhaps a more scientific way to define the epoch is by the onset of the most important cause of the global human footprint: agriculture. "Rather than focus on greenhouse gases, we should identify when in the archaeological record humans first developed a significant ability to modify the Earth's ecosystems," Smith said in a talk co-written with fellow NMNH archaeologist Melinda Zeder. Agriculture arose nearly simultaneously in the Near East, Asia, and the Americas, and "is the lever that humans have used" to refashion the planet, Smith said.

The origins of agriculture about 11,500 years ago coincide with the beginning of the Holocene, which already has an official golden spike—the end of the last ice age. If the marker was early agriculture, no additional spike would be needed, and the current epoch could be called the Holocene, the Anthropocene, or both, Smith said.

Support for using agriculture as a dividing line comes from the dramatic impacts made by early farmers, described in the case of East Asia by archaeologist C. Melvin Aikens of the University of Oregon. Beginning about 9000 years ago, villages fed by millet, wheat, and barley fields began sprouting all over China. This was much more than just a few clearings in the forest: Early rice farmers carved out landscapes and waterways, and "over the millennia vast reaches of southern China were wholly transformed by dams, canals, and vast paddy fields," Aikens said.

Even regions once thought to be relatively untouched by humans, such as the Amazon, were radically changed by prehistoric peoples, who built extensive earthworks and causeways at least 4000 years ago (*Science*, 29 August 2008, p. 1148), panelists noted.

Whatever agriculture's impacts, using it to define the Anthropocene may well encounter resistance from earth scientists. Indeed, in a 2011 article in the *Philosophy*

*Society for American Archaeology 78th Annual Meeting, 3–7 April, Honolulu.

ical *Transactions of the Royal Society A*, a team led by climate change expert Will Steffen of the Australian National University in Canberra, argued that warming of the planet is a key feature of the Anthropocene and that increases in greenhouse gases due to early farming were too small to trigger climate change. The authors also argued against tying the Anthropocene to megafaunal extinctions, claiming that the megafauna had little “appreciable impact on the func-

tioning of the Earth system as a whole.”

Would moving the start of the Anthropocene back into prehistory blunt the epoch’s impact? “When originally proposed, this was a strategy for getting the public to appreciate the extent to which humans were destroying the world,” Smith noted in the question and answer session. But Zeder said that if the Anthropocene does become official in 2016 as proposed, it should be based on solid science. “We are backing away from

coming up with arbitrary markers, and asking what are the human and environmental interactions that are driving it.”

Geologists are apparently willing to hear such ideas. The new journal, *Anthropocene*, plans a special issue on archaeological perspectives, largely drawn from the Honolulu talks, says editor-in-chief and geomorphologist Anne Chin of the University of Colorado, Denver.

—MICHAEL BALTER

EXOPLANETS

Kepler Snags Super-Earth-Size Planet Squarely in a Habitable Zone

The Kepler spacecraft has achieved plenty of milestones in the 4 years and counting that it’s been orbiting the sun. Kepler team members have reported the discovery of ever-smaller exoplanets—down to Earth-size and below—and more and more exoplanets that are in the habitable zone, where a solid, Earth-size planet could have liquid, life-sustaining water on its surface.

Now, the Kepler team is reporting the discovery of what could be the Holy Grail of exoplanetology: a rocky planet enveloped by a nurturing, warming atmosphere and sporting streams, lakes, and seas. Or maybe not. Team members point out in their paper, published online this week in *Science* (<http://scim.ag/WJBorucki>), that they don’t know whether their new find has water, an atmosphere, or even a solid surface. Indeed, no current, planned, or perhaps even conceivable mission could show that this particular exoplanet is habitable.

None of that is Kepler’s fault. The spacecraft’s only job, after all, is to determine how often Earth-size planets orbit in the habitable zone of sunlike stars. And it has already discovered more than 100 new exoplanets down to the size of Earth’s moon. It has done that by staring year after year at more than 100,000 stars, waiting for the few hundredths of a percent dip in a star’s brightness that signals that a planet is orbiting across the star’s face.

Kepler’s latest find is a system of five planets orbiting a star designated Kepler-62.

The planets range from half the size of Earth to twice its size in terms of diameter. Their “years” range in length from 6 days—no doubt making that planet too hot for life—to a more promising 9 months for the system’s outermost known planet, called Kepler-62f.

Although transit observations yield precise measures of an exoplanet’s size and orbital distance from its star, determining



Imagination run free. Exoplanet Kepler-62f’s rising star and neighboring planet (bright point) are known; the presence of clouds, land, and sea are purely speculative.

whether it actually falls in a star’s habitable zone requires some big assumptions. Calculating how much of its star’s radiant energy an exoplanet intercepts is straightforward enough. But what happens next to that energy depends on a host of characteristics of the exoplanet, none of which is usually known.

The big unknown is the nature of an exoplanet’s atmosphere. The Kepler team follows convention and assumes that the exoplanet has a greenhouse atmosphere of nitrogen, carbon dioxide, and water. The habitable zone—the range of orbital dis-

tances within which this greenhouse would keep working—would extend from an outer boundary beyond which carbon dioxide freezes out to an inner boundary within which the greenhouse would overheat, as may have happened on Venus.

In the case of the Kepler-62 system, only Kepler-62f—super-Earth-size at 1.41 times the size of Earth—falls in this strictly defined habitable zone, but it is well within it. Kepler-62e, slightly closer to its star than 62f, falls outside the strictly defined habitable zone but inside a more loosely defined one. Two other relatively small exoplanets in other systems fall in their stars’ habitable zones, but their sizes are not known, only their minimum masses; they could be super-Earth-size or much larger.

Kepler’s discovery of this possibly habitable exoplanet is another grand achievement for the mission, says exoplanet researcher Sara Seager of the Massachusetts Institute of Technology in Cambridge. But she’s worried about the public’s perception of the discovery. “Kepler has achieved great milestones,” she says, but its mission is “not to say ‘that’s habitable, let’s go there.’ Kepler’s stars are too faint; the amount of information will be too low to demonstrate habitability.”

To characterize the atmospheres of potentially habitable exoplanets, astronomers need systems nearer Earth with brighter stars. The Transiting Exoplanet Survey Satellite (TESS) mission approved earlier this month by NASA for a 2017 launch will find those brighter systems, Seager notes. Still, fully characterizing TESS’s exoplanets will challenge even NASA’s next great orbiting observatory, the James Webb Space Telescope, scheduled for launch in 2018.

—RICHARD A. KERR

CREDIT: NASA/JPL-CALTECH/T. PYLE

BIOMEDICINE

Rare Cancer Successes Spawn 'Exceptional' Research Efforts

Most experimental cancer drugs that reach clinical trials fail to help enough patients, and the drugs are shelved. But in many trials, there are the exceptions: a rare patient with advanced cancer whose tumors shrink or even disappear for many months or years. Researchers have typically paid little heed to such "outlier" cases, but that could soon change.

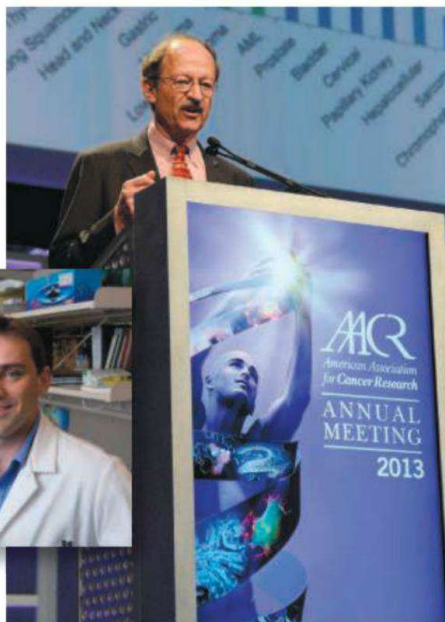
This month, the U.S. National Cancer Institute (NCI) announced that it is casting a wide net for such "exceptional responders" in hopes of ferreting out genetic or other molecular peculiarities that may explain why a drug sometimes has dramatic beneficial effects in certain patients. Identifying such biomarkers underlying sensitivity to a drug could allow more people to benefit from it, agency officials say. NCI also intends to launch a new kind of clinical trial that will genotype a patient's tumor and match it to a potentially effective drug, including experimental drugs that haven't been approved.

"We want to find ways to accelerate movement towards precision medicine," or personalized cancer treatments, says NCI Director Harold Varmus, who highlighted the two projects in an address last week at the annual meeting of the American Association for Cancer Research (AACR) in Washington, D.C. But focusing on rare, exceptional patients may draw skepticism from some, predicts researcher Charles Sawyers of the Memorial Sloan-Kettering Cancer Center (MSKCC) in New York City, who is helping NCI organize the projects: "Can we really learn from single patients? Is this a good way to do science?"

One reason that NCI became interested in what researchers are calling "n of 1" studies is a report last year from MSKCC researcher David Solit. His team observed that everolimus, a new type of cancer drug, didn't help most patients in a trial for advanced bladder cancer, yet one 73-year-old patient went into a remission that has lasted more than 3 years. Her tumor didn't have mutations in genes previously linked to everolimus sensitivity, so Solit's team sequenced her entire tumor genome—the first whole genome sequence

MSKCC had done. This revealed that the woman's tumor carried a mutated version of a gene, *TSC1*, that Solit's group later found in tumors of several other patients in the same trial who responded to the drug (*Science*, 12 October 2012, p. 221).

Solit's group has since studied another exceptional responder: a woman with cancer of the ureter who has apparently been cured by a combination of chemotherapy and an experimental drug that interferes with DNA repair. The combination didn't show promise in a phase I safety trial, leading the company developing the drug to abandon it. But whole genome sequencing revealed that the



Rare finds. NCI chief Harold Varmus outlines plans to find "exceptional responders" to cancer drugs, as David Solit (*inset*) already has twice.

woman's tumor had a mutation in *RAD50*, a gene that codes for a protein involved in DNA repair. (Together, the drug and mutation apparently thwart DNA repair so much that it tips rapidly dividing cancer cells to self-destruct). About 4% of cancer patients have mutations in genes coding for proteins in the same DNA repair complex, suggesting that many others could benefit from the "failed" drug combination. "That's what's so cool about this. You get clues," Varmus says.

At the AACR meeting, James Doroshow, NCI deputy director for clinical and translational research, appealed to the research

community to help find 100 such exceptional responders—defined as a response lasting at least 6 months in a clinical trial for a drug that was not approved for that cancer because too few patients overall responded. If tumor tissue is available and researchers can obtain the patient's or family's consent, NCI will submit the samples to its cancer genome centers for sequencing of protein-coding DNA and other tests. (One challenge is that most archived tumor samples are preserved with formaldehyde, rather than frozen, which limits the possible analyses.) "It's really a data mining, hypothesis-generating exercise," Doroshow says. "We will find some new insights about how drugs work," and "maybe some drugs will be resurrected."

Flipping that concept around, NCI also wants to launch a trial that goes from genotype to phenotype—NCI will analyze a patient's tumor for cancer-spurring mutations and match it with a targeted drug that might shrink the tumor. By mid-2014, the agency hopes to begin enrolling 1000 patients with various types of cancer who have failed standard treatment and test their tumors for 100 or more "actionable" mutations. NCI aims to work with companies to assemble a pharmacy of dozens of gene-targeted drugs that will include ones that have passed safety testing but are still under investigation and not approved for clinical use. (Instead of having a separate control group, NCI may compare tumor growth for a patient on and off the drugs so that each patient is his or her own control, Doroshow says.)

Not everyone thinks that this MATCH trial makes sense. Because many of the mutations will be very rare, it may be difficult to find more than one or two patients in 1000 who have them, Solit says: "You need a certain number of patients to make an interpretation." But others say that as researchers splinter tumors into smaller and smaller subsets using genetic characteristics, it may no longer be possible to launch a traditional trial with hundreds or thousands of patients having the "same" cancer. "If the evidence of clinical benefit is so great, then a few cases may be enough to convince people," says lung cancer researcher William Pao of Vanderbilt University in Nashville.

The trial could also help solve the problem of giving patients access to the drug they need, Sawyers says. Often that happens only if their physician knows how to get a single-patient investigational trial approved and if the company contributes the drug, he says. "More and more of these cases are clearly going to happen, and it's time to have an organized solution," Sawyers says.

—JOCELYN KAISER



CCEMC GRAND CHALLENGE:

INNOVATIVE CARBON USES

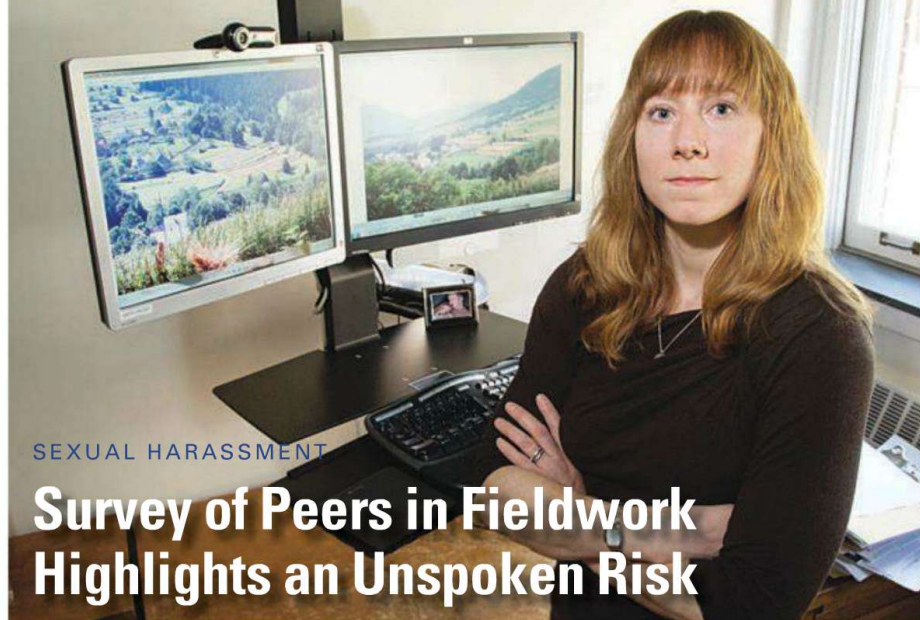
A GLOBAL QUEST TO TURN CARBON INTO A VALUED RESOURCE.

The solution to the world's carbon challenge seems daunting; as the world grows, so does the demand for fossil fuels.

The time to act is now. The CCEMC Grand Challenge is an Alberta initiative to find and support bold ideas from around the world that will make significant reductions in greenhouse gas emissions. We're committing up to CAD\$35 million in funding for the most innovative technology that will convert CO₂ emissions into new carbon-based products and markets.

Find more information and submit your proposal at ccemcgrandchallenge.com





SEXUAL HARASSMENT

Survey of Peers in Fieldwork Highlights an Unspoken Risk

It was standing room only at 8:15 a.m. last Saturday in a session on ethics at the annual meeting of the American Association of Physical Anthropologists (AAPA) in Knoxville, Tennessee. The talk had nothing to do with traditional problems facing anthropology, such as respecting cultures or sharing data. Instead, it focused on how researchers treat each other. The speaker, Kathryn Clancy, a bioanthropologist at the University of Illinois, Urbana-Champaign, had troubling data to share: A survey of her colleagues' fieldwork experiences revealed a high incidence of sexual harassment. The abuses ranged from subtle gender exclusion to sexual assault. The victims were mostly female, and the perpetrators were mostly senior researchers, sometimes the victim's own fieldwork mentor.

"I am shocked, angry, disillusioned, and sad," outgoing AAPA President Lorena Madrigal wrote to *Science* in an e-mail. "I just thought this did not happen anymore, and I am still in shock to hear that it does."

The idea for the survey took shape in 2011, Clancy says, when she learned that a friend had been raped in the field by a colleague and that a mentor convinced the victim to keep quiet for the sake of her career. "It was like a slap in the face," Clancy says. It was unlike her own Ph.D. fieldwork, which she describes as "paradise."

Her friend had opted for silence, but not Clancy, who has a powerful megaphone—she writes a blog for *Scientific American*. Starting in January of last year, she started posting anonymized sexual harassment horror stories that female colleagues shared with her. Anonymous comments started rolling in from fellow scientists. Clancy then teamed up with three of her bioanthropology colleagues—Katie Hinde of Harvard University; Robin Nelson of the University of California,

Riverside; and Julianne Rutherford of the University of Illinois, Chicago—and launched an online survey asking colleagues to share their fieldwork experiences.

At the AAPA meeting, Clancy presented the first month of survey data, based on responses from 98 women and 23 men. Women were far more frequently the target of inappropriate sexual comments, 63% compared with 39% for men. Most troubling, 21% of women reported that they had experienced "physical sexual harassment or unwanted sexual contact." One of the 23 men did as well. Most of the reported abuse happened within the team of researchers, usually perpetrated by someone higher in the professional hierarchy.

The true prevalence of abuse in the field is unknown, because the survey respondents were self-selected. But John Hawks, an anthropologist at the University of Wisconsin, Madison, who attended Clancy's talk, points out that male and female respondents witnessed the same reality. "Male students in the field had exactly the same distribution of perceptions about the presence and severity of abuses as did females," he says.

The reaction to the survey has been overwhelmingly supportive, Hawks says, but there are concerns about its impact. "I spoke to some very senior people in the field who are worried about how making this stuff public will damage public perceptions," he says. But "it is time to do something about this problem." Hinde points out that while U.S. laws prohibit sexual and racial discrimination, a victim may hesitate to take action. "Quitting a field site, not completing

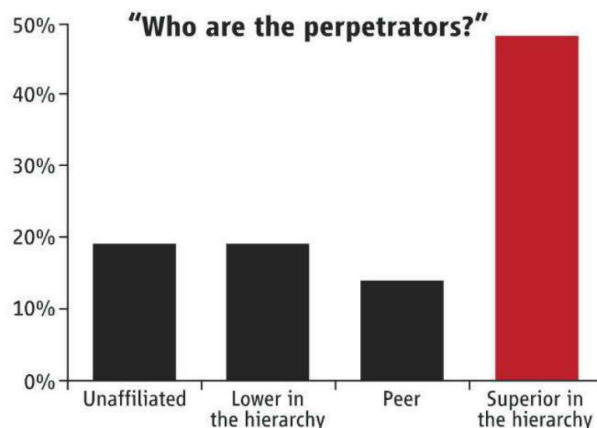
Speaking out. Bioanthropologist Kathryn Clancy and colleagues asked about fieldwork experiences.

and publishing research ... can have potent consequences for academic careers," Hinde writes in e-mail.

Clancy's team is expanding its survey to researchers in other areas of science. "I have had people from nonbioanthro field sites contact me," she says. "This is definitely not limited to just my discipline."

Researchers in other fields say that they have run into similar problems. One suggests a simple remedy: "It seems that having a woman in charge of a field site has a positive effect," says Mary Albert, a veteran earth scientist at Dartmouth College. Albert, who has spent months at remote sites drilling ice cores, says that she has been sexually harassed and groped twice by senior scientists. "But never in the field. In my experience, sexual harassment is worse in the office." She adds that she started going into the field only as a senior scientist.

The road ahead may be difficult for bioanthropology, a traditionally male field where women are flooding in. Research has shown that sexual harassment is more likely to occur in an environment where females "are occupying typically male gender roles," says Sandy Hershcovis, a psychologist at the



Harassment survey. Responses from 98 women and 23 men show that superiors may often be to blame.

University of Manitoba in Winnipeg, Canada. "Men use sexual harassment to exert control, knock them down, or put them in their place." It's rarely about sex or attraction, she says.

Clancy's survey is ongoing and has expanded to other areas of science that involve fieldwork. If you are a field scientist from any discipline, you can take the survey at <http://bit.ly/fieldexp13>.

—JOHN BOHANNON



Trachea Transplants Test the Limits

More than a dozen ill people have received a bioengineered trachea seeded with stem cells during the past 5 years, but outcomes are mixed, and critics say the treatment may not do what its developers claim

FIVE YEARS AGO, CLAUDIA CASTILLO WAS struggling to breathe. A severe case of tuberculosis had damaged the 30-year-old Barcelona mother's airway so much that she couldn't walk up stairs, much less keep up with her two young children. Out of other options, she agreed to help make medical history when her surgeon offered her a radical proposal. In June 2008, she became the first patient to receive a trachea transplant that made use of her own stem cells—the most advanced example at the time of tissue engineering.

The operation, she told various media in the following months, gave her back a normal life. It also made medical celebrities of the surgeons who developed and implanted the artificial trachea: Paolo Macchiarini, Martin Birchall, and their colleagues. They were hailed as pioneers leading the world toward an amazing future of regenerative medicine in which doctors will make replacement parts to order. Since then, 14 other patients have received bioengineered tracheas.

However, almost all the cases so far have been done under “compassionate use” rules, in which untested procedures are allowed in

seriously ill patients for whom conventional treatments are unlikely to help. That makes the outcomes more difficult to evaluate than if the procedures were performed as part of clinical trials. Indeed, some observers wonder if the clinical feats are really examples of successful tissue engineering that utilize the special abilities of stem cells—or an elaborate temporary fix that is destined to fail. More worrisome is that at least two recipients of these bioengineered tracheas have died under circumstances that that might be related to their transplants.

“The motivation is more than noble,” says Harald Ott, a surgeon at the Massachusetts General Hospital Center for Regenerative Medicine in Boston. “The surgeons are trying to help the patients.” However, data from animal studies using the technique are sparse, he and others note. “If you translate something [to patients] very quickly, you don't have the luxury of getting the readout of what's really happening. ... That's why we do animal studies, so that we can see what happens inside the grafts.” In fact, one critic says it is impossible that the trachea implants are working as their makers propose, with

Pushing forward. Paolo Macchiarini has treated more than a dozen patients with bioengineered tracheas.

new cells taking over the structure and creating living tissue. “The claims are hot air,” says Pierre Delaere, a head and neck surgeon at the University Hospital Leuven in Belgium who has written two letters to *The Lancet* criticizing the work.

Both Macchiarini and Birchall are trying to address the doubts about their approach. Now working independently, each has a major grant to conduct traditional clinical trials of stem cell-laden trachea scaffolds, one in Russia and another in the United Kingdom. And Macchiarini, who has won more than \$10 million in grants from the European Union and Russia, has received U.S. Food and Drug Administration (FDA) approval to conduct the first such transplant in the United States.

But Delaere, Ott, and others contend that, before pushing ahead with the clinical trials, the trachea pioneers should focus on understanding what happens to the grafts over the long term. Failing to do so could endanger patients and ultimately damage the field, they say. Although these initial operations have been an important step forward, “they've got a lot of work to do to make a procedure that is available widely to patients,” says Alan Trounson, a stem cell researcher and president of the California Institute for Regenerative Medicine in San Francisco. “In the end, do we have tissue that's going to be functional for the lifetime of the person? I'm unsure whether we really do.”

A delicate balance

Although at first glance the trachea might seem like a simple tube, its thin but cartilage-reinforced walls must stand up to near-constant use as a person breathes, clears his throat, or coughs. Any transplant, therefore, has to be strong enough to withstand such pressures without collapsing. But a rigid prosthesis can rub against and damage the adjacent major blood vessels in the upper part of the chest, leaving a patient at risk for a fatal hemorrhage. At the same time, the natural blood supply for the trachea's tissues is intricate, with vessels too small for surgeons to easily reconnect during a transplant operation. And because it is exposed to inhaled air, the wound between the implant and the remaining airway is especially vulnerable to infection.

Surgeons have tried for years to find ways around these challenges, without much success. When Castillo was hospitalized in Barcelona in March 2008, Macchiarini, who

CREDIT: STAFFAN LARSSON

was then at the University of Barcelona's Hospital Clínic, and Birchall, then at the University of Bristol in the United Kingdom, had experimented with bioengineered transplants in pigs. They would take a trachea from a pig and remove its living cells to create a so-called decellularized scaffold. They seeded this with cells from the recipient pig: bone marrow cells on the outer layer, thought to help form new cartilage, and epithelial cells on the inside, which they hoped would regrow the trachea's lining. They allowed the cells to grow on the scaffold for several days in a bioreactor designed to provide different conditions for the two types of cells. They hoped that the decellularized scaffold would not require immunosuppressive drugs to prevent its rejection and that the seeded cells would take over the removed cells' roles, ultimately forming a living organ.

The pig studies were still unpublished, but the researchers had observed that the animals could survive for 60 days with the implants. Castillo, who was suffering from recurring infections, was facing the possibility of losing one of her lungs. Macchiarini proposed that instead they try to replace part of her trachea using the pig-tested technique, and she agreed.

Birchall readily admits that when the operation took place, "we were a million miles from what we thought we'd need to know before trying it in people." Regulators in both Spain and the United Kingdom, where Birchall's group seeded Castillo's cells on a decellularized trachea from a deceased donor, eventually allowed the procedure without the kind of preclinical testing usually required. "We ran roughshod over regulations—with permission," Birchall recalls. "It wasn't done to the highest possible standards. But it worked, and it changed the way people think about regenerative medicine."

The medical team was aware of the risks, he says. "We had the naïve confidence of youth, I suppose. We believed that it would work. Paolo is a very courageous and skilled surgeon. His was the real courage. My job was to brave the regulatory environment and fit the science to what we needed."

The surgery went smoothly and was soon featured in news stories around the world.

As the researchers reported in *The Lancet* in December 2008, Castillo was released from the hospital without complications after 10 days; several months later, she could again walk several flights of stairs, care for her children, and had normal lung function.

Complications arose a few months later, however, when Castillo's airway narrowed again and collapsed, requiring doctors to insert a stent—a semiflexible tube—to keep her airway open. They removed it 6 months later, Macchiarini says, but she has had to



Transplant success? Claudia Castillo, the first recipient of a bioengineered trachea, with her children several months after the 2008 operation.

have several more inserted and removed over the past 5 years. He says she is now fine and has had her latest stent removed. "Thanks to regenerative medicine, she still has her both lungs and enjoys a superb quality of life," Macchiarini says. "Thanks to her we have learned so much and opened the door for more advanced applications." (Despite multiple attempts to reach Castillo, she did not comment for this story.)

Macchiarini, who moved to the Karolinska Institute in Stockholm in 2010, says that he has treated eight more patients with decellularized and reseeded tracheas, three of them children. In six other patients, he has used a different technique, seeding cells on a synthetic scaffold made of a nanocomposite polymer designed to mimic cartilage instead of a donor trachea.

Not all of the stories have happy endings. One of the children, Birchall says, died of the cancer that necessitated the operation in the first place, although "it gave her quality of life for the last few months." Macchiarini says another adult patient also died of the cancer that had damaged the trachea.

At least two other patients have died suddenly. One was a child who had massive bleeding into her chest after receiving one of the decellularized, reseeded tracheas; "we don't really know what happened," Birchall says. The other, Chris Lyles of Baltimore, Maryland, died on 5 March 2012 after receiving a synthetic scaffold seeded with his stem cells in November 2011 in Stockholm. Lyles had returned to Baltimore in January and was apparently doing well initially. The family did not make his cause of death public, and according to Macchiarini, there was no autopsy, although he suspects pneumonia based on the medical report.

But thoracic surgeon Alain Wurtz of the Lille University Teaching Hospital in France says both cases could also be consistent with the rupture of major blood vessel, which the implant might have caused. (Wurtz has treated several patients who need new tracheas by transplanting part of an aorta from a tissue bank into the neck.)

The researchers have published two case reports in addition to Castillo's. In July 2012, Birchall and his colleagues described in *The Lancet* the 2-year follow-up of the first child to receive a transplant. The paper notes that the boy, whose own trachea

had never developed properly, needed several stents in the months following the transplant, but by 18 months his chest CT scan and lung function were normal and he had returned to school.

In 2011 in *The Lancet*, Macchiarini and his colleagues described the 5-month follow-up of Andemariam Beyene, a geophysics graduate student in Iceland who had a large tumor growing on his trachea. He received the first synthetic scaffold. His current doctor, Tomas Gudbjartsson of Landspítali University Hospital in Reykjavik, tells *Science* that Beyene has had several stents, but is healthy enough that he was able to complete his studies last year. The researchers have mentioned other patients in passing in several papers, but no formal reports have been published about their health, and *Science* has not been able to independently verify the current status of all the patients.

Lingering questions

What's been published and presented at meetings so far hasn't satisfied critics of the stem cell approach. "They should not

tell the world that they did the first synthetic organ transplantation,” Delaere says. Simply wrapping an artificial stent with tissue called omentum is known to be a temporary treatment for patients who need new tracheas and that, in essence, is what has happened in the recipients of the cell-seeded tracheas, Delaere contends. (Macchiarini and his colleagues wrap the transplants in omentum to reduce inflammation and promote blood vessel growth.) “If they claim something miraculous, they have to show corresponding data. They don’t do that,” says Delaere, who has developed his own solution to damaged tracheas: taking an intact trachea from an organ donor and implanting it in the patient’s forearm for a few months, allowing new blood vessels to grow before it is transplanted into the neck. *The Lancet* reports, he charges, do not provide convincing evidence that the graft is functioning properly. Wurtz agrees: “There is absolutely no detailed data about these patients,” he says.

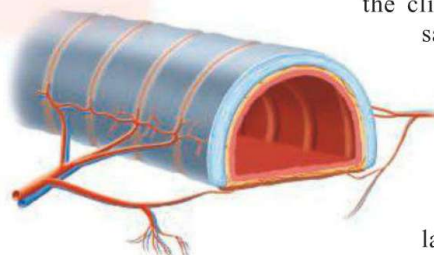
Macchiarini and Birchall, who is now at University College London, acknowledge that it is difficult to tell what exactly has happened inside the implanted tracheas. They say, though, that clinical outcomes are their primary concern and the current stable health of more than half of the recipients is strong enough evidence that the technique works. Both are now working on clinical trials of the technique, although they are no longer working together, in part because of a dispute over the contents and authorship of one of the transplant papers.

In March, Birchall received a £2.8 million (\$4.3 million) grant from the United Kingdom’s Medical Research Council to conduct a trial of decellularized and stem cell-seeded upper trachea and larynx, with roughly 10 patients. Macchiarini has already completed two transplants in Russia as part of a clinical trial—funded with a \$6 million grant from the Russian government—that he says should eventually enroll 20 or 25 patients. “We were allowed to do this type of transplantation only in extreme cases,” he says. “The clinical study for the first time gives us a chance to include patients who are not in such critical shape.”

Macchiarini is also the lead investigator on a 5-year, €4 million (\$5.2 million) grant from the European Union to begin a clinical trial using decellularized tracheas and further develop the polymer scaffolds in large animal models. That project may need to be reorganized, however, following a legal dispute last year in Italy, where the transplants were supposed to take place—



Not so simple. The trachea extends from the larynx to the chest and is formed from a thin membrane supported by cartilage rings and sustained by blood vessels.



Macchiarini had a part-time position at Careggi Hospital in Florence. In September, however, Italy’s financial police accused him of attempted extortion, and briefly placed him under house arrest, for allegedly telling a patient that he could receive treatment in Germany for €150,000. Macchiarini and his lawyer say that he was simply informing the patient of possible options, not demanding payment. The main charges were soon dropped, but Macchiarini says that the charges stemmed from academic politics in Tuscany and he has severed ties with the hospital and university there. “There is no way to go back there.”

A solid base?

The questions that remain 5 years after Castillo’s transplant are highly relevant for the field of tissue engineering as a whole. When other researchers have used cell-seeded scaffolds to repair blood vessels, they have shown that it is not the seeded cells that ultimately populate the graft. Rather, the seeded cells produce signals that both dampen the body’s inflammation response to the transplant and attract other cells from surrounding tissue. These immigrants ultimately take up residence and form the new tissue (*Science*, 26 August 2011, p. 1088).

No one knows whether something similar is happening in these engineered tracheas. The detailed analysis of the vascular implants was conducted in mice because it’s relatively easy to genetically tag mouse cells

and trace where they end up. Mouse tracheas, however, are extremely small, making surgery impractical. Most of the animal studies on trachea replacement have been in pigs, sheep, and rabbits, and most researchers have not attempted to find out exactly how the replacement trachea is repopulated.

That is a missed opportunity, says Christopher Breuer, a pediatric surgeon at Nationwide Children’s Hospital in Columbus. Breuer has used cell-seeded grafts to repair severe heart defects in children and led the work in mice that demonstrated the details of how the grafts work. “I think it is important to take a step back and look at what is happening” in animal models before

the clinical trials start, he says. “Ultimately, the half a step back will allow you to take many steps forward.”

Macchiarini has done additional lab-based experiments to find out what happens to decellularized tracheas over time. After a year in saline solution, he and his colleagues found, human tracheas became significantly weaker and lost some of the microstructure that is thought to help new cells take up residence and regenerate healthy tissue. “It’s not surprising. That’s physics in action,” says Doris Taylor of the Texas Heart Institute in Houston, a co-author on the study, which was published in *Biomaterials* last year. In the body, incoming cells probably mitigate the effect, she says, but the details are not clear.

Those results, and the collapsing airways of Castillo and other initial transplant recipients, were what prompted Macchiarini to switch to the synthetic scaffold, developed by materials scientist Alexander Seifalian at University College London and other researchers. The Russian clinical trial is using the lab-made scaffolds, and this month Macchiarini is scheduled to implant one in a child for the first time. The patient is a 2-year-old born with an undeveloped trachea. FDA has given its approval under its Investigational New Drug rules, which allow for exceptions to full approval.

But even controlled clinical trials won’t reveal as much as well-designed animal studies, Ott says. That means that it is difficult for other groups to build on what has been published, he says. Taylor concurs. “We are never going to understand everything,” she says. “But we have to understand enough to make it reproducible, safe, and effective.”

—GRETCHEN VOGEL

CREDIT: PIERRE DELAERE (2)

PROFILE: LAURENT KELLER

Chasing Ants—and Robots—to Understand How Societies Evolve

Laurent Keller's passions go far beyond ants as he taps genomics, robots, and other approaches to answer evolutionary questions

LAUSANNE, SWITZERLAND—Kenneth Ross still vividly recalls the day that Laurent Keller saved his life. Ross, an entomologist at the University of Georgia, had been so focused on looking for fire ants in rural Georgia that he stepped into the road just as a logging truck roared by. Fortunately, Keller was quick enough to grab Ross out of the truck's path. "I can still smell the pine rushing past," Ross says of the 1993 incident.

Keller's quick reflexes and decisive actions have held him in good stead in his science as well. At the time, Keller was a young Swiss postdoc with poor English skills but a lot of enthusiasm. Twenty years later, at the University of Lausanne, he has more than 280 publications that testify not only to his language proficiency but also to his ability to jump quickly into emerging fields and make early critical insights. "He always has a good feel for where a field is heading and is always at the forefront," says Jürgen Liebig, a behavioral ecologist at Arizona State University, Tempe. That, coupled with persistent attention to key problems, has led Keller, 52, to success on multiple levels, including receiving the E. O. Wilson Naturalist Award in 2005 from the American Society of Naturalists.

Almost 15 years ago, Ross and Keller came up with one of the first genes underlying social behavior. Just a few months ago, Keller's team reanalyzed that "gene" and showed that it is actually a large segment of inverted DNA that encompasses 600 genes. In the meantime, Keller delved into social conflict, crafting experiments that shore up key theoretical ideas about how societies work. He also teamed with engineers and computer programmers to build robots that "evolved" social behavior. That work "planted the seeds for a whole new approach

to understanding social evolution," says Gene Robinson, an entomologist at the University of Illinois, Urbana-Champaign. Most recently, Keller's lab has shifted into studies of collective behavior in ants, using a newly



Social scientist. Laurent Keller is serious about his research on social behavior in insects, but colleagues say he also is fun to be with.

developed tracking system that can follow up to 225 individual ants 24/7 for weeks at a time (see sidebar, p. 270). "We try to do stuff that [other] people don't," Keller says.

Keller "has a knack of picking important questions, picking the right system, and having the persistence in chasing them," says Andrew Bourke, an evolutionary biologist at the University of East Anglia in the United

Kingdom. And his work has relevance far beyond ants. "Understanding how individual behavior is specified in large groups [can lead] to principles of social organization that may explain the organization of other groups, including humans," says University of Lausanne neurobiologist Richard Benton. In ants, unlike humans, "you can test your hypotheses about how communication between individuals is going to affect the group as a whole," Benton adds.

Tenacity

As an undergraduate at the University of Lausanne, Keller knew he wanted to study social behavior in animals. Great apes were his first choice, but he realized that there would be a low return per hour of fieldwork. Zoo studies present an artificial environment. So he settled for ants.

He became fascinated with fire ants, South American invaders that have spread rapidly across the southern United States in the past 80 years. In particular, he wanted to understand why some fire ant colonies support a lone queen, while others form supercolonies with multiple queens.

He struck up a close collaboration with Georgia's Ross, at one point serving a joint postdoctoral fellowship with Ross and Harvard University's famed ant researcher Edward O. Wilson. Several times during the late 1980s and 1990s, Ross and Keller traveled to Latin America, spending weeks on the road crisscrossing thousands of kilometers in search of native populations of fire ants. More than once, Keller, in his faulty Spanish, negotiated their way out of sticky situations—gauchos riding up with guns drawn, or border police trying to shake them down for bribes. He explained to all comers that they were legitimate researchers and must continue their work—and he got his way. "He's like a bulldog, he doesn't

give up," Ross says. "He does the same thing with his science—he's tenacious."

One example of that tenacity is his and Ross's 15-year quest to understand the genetic basis underlying the multiqueen fire ant colonies. In 1997, lacking the technology to look for DNA differences, Ross had fingered a protein called Gp-9 that seemed to differ in ants from the two types of colonies.

In 1998, he and Keller showed that the still unidentified gene for Gp-9 had both a dominant and a recessive version, or allele. Workers with both versions of the allele accepted multiple queens, provided those queens also had both alleles. Otherwise the workers killed the extra queen.

Gp-9 is an odor molecule found all over an ant's surface. Yet, the different versions of the gene seemed to also determine worker behavior, the colony's social organization, and even the size of the queen. "It started to become difficult to think that it was a single gene for all of those traits," Keller recalls. But at the time, Ross and Keller didn't have the technology to explore further.

Still, they kept the problem in mind. When the revolution in DNA sequencing put the genomes of many organisms within easy reach, Keller acted. In 2008, he helped organize the effort to decipher the genome of the fire ant *Solenopsis invicta*. Published in 2011, the genome paved the way for Keller to find out about Gp-9. Sequencing doesn't physically place genes on chromosomes, so Keller used a technique called RAD-sequencing (*Science*, 25 February 2011, p. 1006), which generates thousands of landmarks on chromosomes, to pinpoint Gp-9. Thus, he could see which genes were linked to it, as those linked genes might also be involved in prompting tolerance of multiple queens.

To his team's surprise, they found a 13-million-base block around the Gp-9 gene that was passed down to offspring as a single unit—essentially a "supergene." During reproduction, matching chromosomes from the male and female usually cross over and recombine to create new mixtures of alleles, but this doesn't happen with this stretch of DNA. As the team reported in the 31 January issue of *Nature*, the block, which contains more than 600 genes, is inverted with respect to the DNA on the matching chromosome, thereby preventing recombination. Thus, each parent's contribution remains unchanged as it passes on to the next generation. "I was surprised it was so big," says Keller, who dubbed the DNA a "social chromosome."

The discovery "opens an entirely new area of research for this system," says Mathieu Joron, an evolutionary geneticist at the National Museum of Natural History in Paris. One challenge now is to determine which of those 600 genes set the stage for multiqueen colonies.

The Private Lives of Ants

Danielle Mersch's ants have no privacy. Six years ago as a graduate student in Laurent Keller's lab at the University of Lausanne in Switzerland, she had wanted to study circadian rhythms in ants but hadn't wanted to spend all day and night keeping track of them. So she and Keller teamed up with Alessandro Crespi, an engineer at the Swiss Federal Institute of Technology in Lausanne to develop a 24/7 surveillance system that promises to revolutionize their study of collective behavior in these insects. "It's groundbreaking technology," says Iain Couzin, a system biologist at Princeton University.

Online this week in *Science* (<http://scim.ag/DPMersch>), Mersch, Crespi, and Keller describe using this system to reveal that ants cluster into cliques: Nurses that tend the queen and brood hang out in one part of the colony, while foragers stick close to the nest entrance, with the two groups rarely interacting. Some organization was expected, but "I was surprised that they have such a strong spatial structure," Mersch says.

The paper's most significant advance, however, may be the setup itself. Mersch struggled to find a suitable marker that would let a computer follow each ant and take data frequently. Many ant researchers paint colors on the ants' abdomens to uniquely identify each one, but colors are hard to incorporate into an automated tracking system, especially one that has to operate in the dark. Radio tags don't provide enough spatial resolution, Mersch says. Other automated trackers record data at long intervals, leaving gaps in coverage.

Mersch settled on a set of 225 barcodes developed for video game developers and glued a barcode to each ant's back; the ants seem to get used to their signboards. High-resolution cameras mounted above the ants' home—a shoebox-sized nest box and foraging area connected by a tunnel—is synced with infrared lights that flash on twice a second. The digital images are immediately relayed to a computer, where software analyzes each ant's position relative to other ants and computes their trajectories. Thus, Mersch can watch ants move in real time or days later.

Ants at a glance. Ants wearing barcodes are tracked by overhead cameras that record the ants' positions in their homes (bottom).

Typically, she starts with 200 ants and has the system observe them for 40 days. So far, she and her colleagues have spied on five species of ants. "It's very important information when you want to investigate the dynamics within societies," says systems biologist Guy Theraulaz of CNRS, the French national research agency, in Toulouse.

About one-third of the colony works with the brood in one corner of the container as nurses; another third forages. Foragers turn out to have a daily rhythm, temporarily leaving the nest by night, but nurses don't. Many of the rest may be housecleaners. Over time, nurses are likely to become housecleaners and, eventually, foragers, moving further out into the world as they age. "There's a clear behavioral trajectory," Mersch says. Next, she plans on manipulating the social structure of the colony, say, by removing all the nurses, to see which ants take their places.

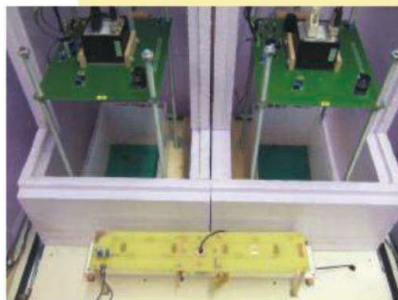
"Until now, biologists have looked at the colony level," Couzin says. "Now they can look at the life history of every individual in the colony. This offers a remarkable way of relating individual properties to collective properties."

—E. P.

Against the grain

But Keller likes challenges. Although he says that he doesn't take himself too seriously, beneath his broad smile and welcoming eyes is an intense, strongly opinionated man who doesn't hesitate to speak his mind or criticize

those around him. "He never seems to get angry, but will state very plainly and in a non-emotional way, 'This is worthless,'" says his postdoctoral fellow Eric Lucas. Keller signs his reviews, so colleagues know whose blunt words helped make or break a paper. "Some



people are rubbed the wrong way,” Ross says.

Several lines of inquiry in his lab have gone against the grain, including work on how insect caste is determined. “He’s really been looking at a variety of different species that get us thinking outside the box about ways to maintain different castes,” explains Andrew Suarez, an ant ecologist at the University of Illinois, Urbana-Champaign.

Researchers had long assumed that environmental factors determined whether a female social insect became a queen or a worker, as both types have the same set of genes. That’s how it works in honey bees. But in the mid-2000s, several exceptions emerged in ants. In each case, Keller’s group or others noticed unexpected genetic differences, for example between queens and males. With such strange data, “most people would have given up,” says evolutionary biologist Daniel Kronauer of Rockefeller University in New York City. “[Keller] noticed what was going on and what the implications would be.”

Through a series of experiments, Keller’s team showed that these ants have an unusual reproductive biology. In one case, for example, workers were sexually produced—a combination of male and queen genetic material. But queens were parthenogenetic and were clones of each other. And the queen’s DNA was discarded in male offspring, making all males clones of each other. “It’s as if you had two species’ genomes that only come together in the sterile worker,” Keller explains.

In these cases, genetics, not environment, determines caste, and Keller thinks that there may be many more such examples among social insects. But he says it’s taken a decade for his colleagues to accept this idea. “Many people are bad scientists,” he says bluntly. “When they get data that doesn’t fit, they do everything they can to throw out the interesting stuff.”

Still, some others suspect that environmental factors, not genetics, are the key to caste most of the time. “It’s up in the air whether [genetic caste determination] is extremely rare or just occasional,” says evolutionary biologist Francis Ratnieks of the University of Sussex in the United Kingdom.

Robotic stand-ins

Some of his ideas defy current wisdom, but Keller, who is well established in an enviable position in Lausanne, is not worried

about controversy. His office looks out on deep blue Lake Geneva, with the Alps as a backdrop. He’s never had a Swiss National Science Foundation grant proposal rejected, and with the money he has in hand right now—including €2.5 million for 5 years

These variations, surrogates for genetic differences, cause the robots to have slightly different behaviors. The researchers score the robots for how well they do retrieving large and small disks, and the most efficient ones are selected to continue and “reproduce”: In each successive round, the researchers increase the proportion of the best robots in the mix, adding clones that have slight variations reflective of what might happen during reproduction.

At first, movements and disk-retrieval are random, but within 150 generations the selection process leads to robots that excel at working together to retrieve large disks, the team reported in 2010. The next year, they demonstrated that the robots even evolve altruism toward their closest relatives. The work showed how easy it is for cooperation to emerge in organisms lacking much of a brain.

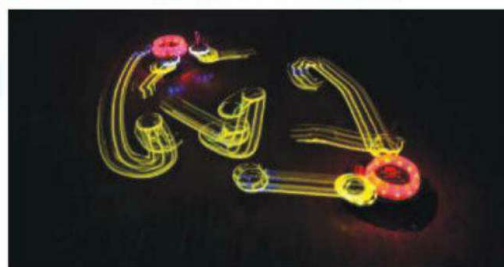
It doesn’t take much of a brain to evolve communication either, according to other work by the team. In these experiments, the robots have the ability to flash blue or red when they encounter “food.” Within 10 generations, different sets of robots adopt different signaling strategies—flashing one color to signal that they have found food, or using combinations of colors to signal food or no food. The simple system is more efficient when there is no competition. But when competing robots try to read the signals, the complex codes are harder to break and thus more useful, the Lausanne team reported on 3 January 2012 in the *Proceedings of the National Academy of Sciences*. “[Keller] showed how a complex strategy can evolve and could beat simpler strategies,” Moses says.

Floreano and Keller are coming up with ever more complicated ideas to test with robots and simulations, but Keller is most excited now about the new ability to follow individual ants in colonies. Even with genomics, robots, and a new tracking system under his belt, however, Keller says he still feels like a rolling stone whose direction is not yet determined. “I hope in 5 years I’m starting to do new stuff that I am not even thinking about today.”

—ELIZABETH PENNISI



Communication 101. In this evolution experiment, robots (glowing blue, above) move (inset) to find the “food” and signal other robots.



from the European Research Council—he can afford to take on risky projects and keep his 15 postdocs and students employed indefinitely. Just 50 kilometers away is his chalet, where he spends winter weekends skiing, sometimes with his two teenage children or members of his lab. Colleagues rave about how fun he is at meetings; several have photos of him dancing on a bar table late one night in Japan.

That solid standing has helped Keller start unusual collaborations. Although his lab is adept at manipulating ants to understand their society, the researchers have been hard-pressed to come up with ant experiments that address how sociality arises in the first place. So a decade ago, Keller went across campus to the Swiss Federal Institute of Technology in Lausanne and teamed up with roboticist Dario Floreano. Together, they and their students harnessed robots and computer simulations to look at the early evolution of cooperation and communication. “The robots are really valuable for understanding limits and constraints on evolutionary processes,” says Melanie Moses, a computer scientist at the University of New Mexico, Albuquerque.

Each robot is built with a slightly different neural network connecting its infrared and vision sensors to its moving wheels.



LETTERS

edited by Jennifer Sills

Drought and China's Cave Species

BETWEEN 2005 AND 2012, A CAVE BIODIVERSITY PROJECT, SUPPORTED BY THE World Bank/Global Environment Facility (1), allowed Chinese and international scientists to survey the fauna of 117 caves in 32 parts of Guangxi Autonomous Region, China. Nearly 10,000 cave specimens and over 500 species were collected, and 150 of these were unknown to science. Most species are limited to a single cave. It is increasingly clear that the cave fauna of southwestern China is a global hotspot of biodiversity (2).

The impact of severe drought in southwestern China appears to be an example of how climate change and poor environmental management can combine to create a biodiversity disaster (3). The impact on cave biodiversity has been overlooked (4). Water resources in caves will decrease during drought seasons (5), especially when people are searching for and extracting water. This will cause the endemic aquatic biodiversity to decline dramatically, or even disappear. Because these species have

generally lost all pigmentation and are often blind, they are incapable of living outside the cave environment. Droughts affect many caves across southwestern China, and each hill or set of hills typically has its own endemic fauna (6, 7).

This part of China and other karst regions in Asia and beyond have exceptionally range-restricted and acutely poorly known faunas, which are under threat from a range of climate change and human actions. We do not want to see a newly revealed global biodiversity hotspot lost before it is known.

SHU-SEN SHU,^{1*} WAN-SHENG JIANG,^{1*} TONY WHITTEN,² JUN-XING YANG,^{1†}
XIAO-YONG CHEN^{1†}

¹State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China. ²Fauna & Flora International, Cambridge, CB1 2JD, UK.

*These authors equally contributed to this Letter.

†Corresponding authors. E-mail: yangjx@mail.kiz.ac.cn (J.-X.Y.); chenxy@mail.kiz.ac.cn (X.-Y.C.)

References

1. T. Whitten, in *Defying Extinction: Partnerships to Safeguard Global Biodiversity*, L. Fitzpatrick, (Earth in Focus, Arlington, VA, 2010), pp. 73–76.
2. T. Whitten, "Discovering a new global biodiversity hotspot in China," *Fauna & Flora International* (January 2013); www.fauna-flora.org/discovering-a-new-global-biodiversity-hotspot-in-china/.
3. J. Qiu, *Nature* **465**, 142 (2010).
4. W. Sutherland, *Trends Ecol. Evol.* **27**, 12 (2012).
5. C. Y. Hu *et al.*, *Int. J. Speleol.* **37**, 143 (2008).
6. T. Whitten, *J. Appl. Ecol.* **46**, 520 (2009).
7. R. Clements *et al.*, *BioScience* **56**, 733 (2006).

In the dark. The *Sinocyclocheilus rhinoceros* lives in the cave of Luoping, Yunnan, China.



Little Emperors Pose Behavioral Challenges

IN RESPONSE TO OUR REPORT "LITTLE emperors: Behavioral impacts of China's one-child policy" (22 February, p. 953), X. Zhao *et al.* argued that only children resulting from China's one-child policy (OCP) are successful relative to their peers ("China's little emperors show signs of success," Letters, 22 February, p. 905). However, our methodologies differ from those of the studies that Zhao *et al.* cite.

The papers cited by Zhao *et al.* rely on a simple comparison of behavioral outcomes between only children and children who have siblings. It is unclear whether the observed behavioral differences (or lack of behavioral

differences) reflect parental behavioral differences or the effect of growing up without siblings. The effect of family background would be even more pronounced in families that had more than one child under OCP; these families would likely live in more rural environments, be minorities, or be immune from OCP through wealth or political connections.

We addressed this issue by comparing people born just before and just after OCP. Our instrumental variables method allows us to compare individuals who grew up as only children because of the OCP and who would have otherwise grown up with siblings, with individuals who were born just before the introduction of the OCP and hence grew up with siblings. We analyzed and controlled for

the possibility of sample bias in education, wages, and age. (More details can be found in the supplementary online materials of our Report.) To clarify the power of our approach, we compared our results with those one would find if one did not account for the endogeneity problem explained

Letters to the Editor

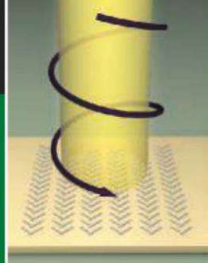
Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

CREDIT: XIAO-YONG CHEN



How black carbon
affects climate

280



Directing surface
plasmons

283

above. In the section on “Evidence of Endogeneity” and in table S5 of our Report, we show that without the instrumental variables approach, we would have found differences between the two groups only in the trust game. However, with our approach, we detected that growing up without siblings also has an impact on risk and competition preferences.

Our Report also differs from previous studies because it uses experimental techniques in addition to survey questions to measure behavioral differences. We conducted games from the economics literature that are designed to elicit altruism, the ability to trust, trustworthiness, risk preferences, and competitiveness, allowing researchers to observe specific, well-defined types of behavior. Monetary incentives within the games were used, as experimental economists argue that they provide greater motivation for participants to reveal their true preferences (1). Our data may thus serve as more reliable indicators of behavior than self-reported surveys.

LISA CAMERON,^{1*} NISVAN ERKAL,²
LATA GANGADHARAN,³ XIN MENG⁴

¹Department of Econometrics, Monash University, Clayton, VIC 3800, Australia. ²Department of Economics, University of Melbourne, Parkville, VIC 3010, Australia. ³Department of Economics, Monash University, Clayton, VIC 3800, Australia. ⁴Research School of Economics, College of Business and Economics, Australian National University, Canberra ACT 0200 Australia.

*Corresponding author. E-mail: lisa.cameron@monash.edu

Reference

1. D. Davis, C. Holt, *Experimental Economics* (Princeton Univ. Press, Princeton, NJ, 1993).

Preventing Prejudice in Genome Profiling

IN THEIR REVIEW (“DIAGNOSTIC CANCER genome sequencing and the contribution of germline variants,” special section on Cancer Genomics, 29 March, p. 1559), O. Kilpivaara and L. A. Aaltonen highlight the potential of whole-genome sequencing to revolutionize cancer research, enabling predictions of oncological predisposition, risk profiling, and in turn, personalized cancer prevention strategies. We agree with the probable significance

of this technology, but believe that it must be implemented with care and caution.

In current U.S. practice, racial inequality in access to health care is well documented (1). This has also been the case in global health. This prejudice extends beyond access to health care; therapy development has largely focused on diseases impacting higher socioeconomic groups, rather than those in resource-limited settings, who frequently suffer from the greatest burden of disease (2). We believe that advancements in genomic profiling may deepen this prejudice, promoting development of therapies more extensively targeted toward lucrative markets and populations. This could result from population biases in existing reference genomic data as well as

from the identification of targeted efficacy in trial cohorts from richer nations (3).

Genomic sequencing—with its potential to identify patient risk and enable the generation of personalized oncological therapies (“Cancer pharmacogenomics: Early promise, but concerted effort needed,” H. L. McLeod, Reviews, special section on Cancer Genomics, 29 March, p. 1563)—must therefore be considered in conjunction with its potential limitations. Advances in this field must be accompanied by appropriate policy development to ensure equity in the collective endpoints of the global pharmaceutical market.

MAHIBEN MARUTHAPPU^{1*} AND
ALEXANDER E. FINLAYSON²

¹Harvard University, Cambridge, MA 02138, USA. ²INDOX Cancer Research Network, Oxford University, Oxford, OX3 7BN, UK.

*Corresponding author. E-mail: maruthappu@post.harvard.edu

References

1. M. E. Gornick *et al.*, *N. Engl. J. Med.* **335**, 791 (1996).
2. S. Kappagoda, J. P. Ioannidis, *Br. Med. J.* **345**, e6512 (2012).
3. M. Ramsay, C. T. Tiemessen, A. Choudhury, H. Soodyall, *Hum. Mol. Genet.* **20**, R214 (2011).

CORRECTIONS AND CLARIFICATIONS

Research Article Summary: “Optogenetic dissection of entorhinal-hippocampal functional connectivity” by S.-J. Zhang *et al.* (5 April, p. 44). The link for the supplementary materials is www.sciencemag.org/content/340/6128/1232627/suppl/DC1. The HTML and PDF versions online have been corrected.

TECHNICAL COMMENT ABSTRACTS

Comment on “*Drosophila* Dosage Compensation Involves Enhanced Pol II Recruitment to Male X-Linked Promoters”

F. Ferrari, Y. L. Jung, P. V. Kharchenko, A. Plachetka, A. A. Alekseyenko, M. I. Kuroda, P. J. Park

Conrad *et al.* (Reports, 10 August 2012, p. 742) reported a doubling of RNA polymerase II (Pol II) occupancy at X-linked promoters to support 5' recruitment as the key mechanism for dosage compensation in *Drosophila*. However, they employed an erroneous data-processing step, overestimating Pol II differences. Reanalysis of the data fails to support the authors' model for dosage compensation.

Full text at <http://dx.doi.org/10.1126/science.1231815>

Comment on “*Drosophila* Dosage Compensation Involves Enhanced Pol II Recruitment to Male X-Linked Promoters”

Tobias Straub and Peter B. Becker

Conrad *et al.* (Reports, 10 August 2012, p. 742) reported that *Drosophila* dosage compensation might largely be due to increased recruitment of RNA polymerase II to promoters. A reassessment of the numerical operations revealed that the authors' calculations are severely confounded by an inappropriate numerical procedure. A rectified analysis strongly suggests that the authors' conclusions are not supported by their data.

Full text at <http://dx.doi.org/10.1126/science.1231895>

Response to Comments on “*Drosophila* Dosage Compensation Involves Enhanced Pol II Recruitment to Male X-Linked Promoters”

Juan M. Vaquerizas, Florence M. G. Cavalli, Thomas Conrad, Asifa Akhtar, Nicholas M. Luscombe

Ferrari *et al.* and Straub and Becker wrongly claim that an error in the computational analysis calls into question the conclusions of Conrad *et al.* All the available evidence, including the reanalyzed genomic data, shows that the conclusions and the key message of the study remain unchanged: RNA polymerase II recruitment to male X-linked promoters is an important regulatory step during dosage compensation.

Full text at <http://dx.doi.org/10.1126/science.1232874>

GEOPHYSICS

Did You Feel It?

Gregory C. Beroza

The grip that earthquakes have on our imagination is expressed by the penetration of seismological vocabulary into wider use. Extreme events—if they're sufficiently disruptive—are described as “like an earthquake.” “Seismic” is used as an emphatic synonym of “significant.” The point of origin of an unfortunate event is referred to as “the epicenter.” The worst conceivable disasters are described as “the Big One.” At their worst, earthquakes are the most extreme, sudden-onset disasters humankind has had to deal with. Nothing else has caused such devastation on such a large scale so quickly. Earthquakes are yet more unnerving because they refute the notion of terra firma, occur without warning, and progress from barely perceptible to violent in seconds. Fortunately, large earthquakes occur infrequently. But that makes them unfamiliar to those who have the misfortune to be caught in them, difficult to prepare for, and challenging to study.

The Earthquake Observers traces the emergence of seismology from the aftermath of the 1755 Lisbon earthquake through the development of the local magnitude scale in 1935. Historian Deborah Coen (Barnard College) draws on a surprising number of luminaries who have commented on earthquakes or used them as metaphors for other events. These include the usual suspects (e.g., Alexander von Humboldt, Charles Darwin, John Muir, and Mark Twain) already well known to seismologists but also others (e.g., Immanuel Kant, Charles Dickens, Friedrich Nietzsche, Ernst Mach, and Karl Popper) whose intersections with earthquakes are less familiar. In documenting the history of early seismic observations in Scotland, Switzerland, Austria, and

California, the author demonstrates how the approach, and even the goals, of earthquake science are intertwined with and influenced by their historical and political context. The book is well written, the documentation meticulous, and the depth of research impressive. At many points in the narrative, I marveled at the extent of the relevant material Coen has unearthed.

The book's central theme is that seismology began as a broadly participatory enterprise, with the public providing essential information on the location and character of earthquakes, but in its shift to a quantitative field driven by instrumentation and analysis, something important

was lost. Although Coen focuses on seismology, she also touches on other highly relevant efforts, such as understanding weather, where citizen-scientists have played important roles. The chronology of attempts to recruit amateur earthquake observers that Coen assembles to make her case is fascinating, and on that basis alone the book is worth reading. Seismologists will recognize the origins of tensions in the field that exist to this day, such as the distinction between regional and teleseismic earthquake monitoring, in the early development of the field.

Coen chronicles many attempts of observers to document the direction of approach of seismic waves and the direction of shaking

during an earthquake as clues to the quake's point of origin and physical mechanism. The futility of these efforts, given the variety of seismic waves with differing polarizations, is obvious to seismologists (in retrospect), and I found reading about these earnest attempts interesting but a bit painful. In recounting them, the author (perhaps unwittingly) makes the case for an instrumental approach.

It's natural that scientists seek to quantify earthquakes to the extent possible, and instrumentation is essential to that effort. In the early 19th century, the technology to record ground-shaking with fidelity did not exist. Until the dawn of the 20th century, accounts of eyewitnesses and the effects of earthquakes on structures were all that was available. As instrumentation improved to the point where quantitative analysis of seismograms was possible, it was inevitable that the emphasis of earthquake science would shift, and we're better informed as a result. Advances in instrumentation underlie most of what we have learned about earthquakes—from locations precise enough to define the three-dimensional geometry of faults to quantification of the intensity of shaking used for earthquake-resistant design. Recent discoveries, such as the diversity of slow earthquakes, continue to drive the field in unanticipated directions and are only possible through improved instrumental observing systems.

That's not to say that there isn't an important role for public contributions of the sort that Coen extols. There are, after all, billions of people and only thousands of earthquake-monitoring sensors. Internet technology lets researchers recruit help from interested citizens on a massive scale. For example, the U.S. Geological Survey's Did You Feel It? website (1), which gathers reports of shaking, received over 77,000 responses for the

2010 El Mayor-Cucapah earthquake. Data from the system have demonstrated that the crust in the eastern United States transmits seismic waves more efficiently than in the West and even revealed the signature of postcritical reflections off the Mohorovicic discontinuity in people's perceptions of the strength of shaking (2). The Internet has also enabled a new hybrid citizen-expert model in which telemetry from volunteers and inexpensive sensors embedded in, or connected to, personal com-

The Earthquake Observers
Disaster Science from
Lisbon to Richter

by **Deborah R. Coen**
University of Chicago Press,
Chicago, 2013. 356 pp. \$35,
£24.50. ISBN 9780226111810.



Dramatized depiction. “Earthquake wave at Lisbon,” the frontispiece from (4).

The reviewer is at the Department of Geophysics, 397 Panama Mall, Stanford University, Stanford, CA 94305–2215, USA. E-mail: beroza@stanford.edu

puters are used to form earthquake monitoring networks (3). The author briefly mentions *Did You Feel It?* but does not highlight the return of citizen science that it represents, as I expected she might—perhaps because her account ends with the introduction of Richter's local magnitude scale in the 1930s.

Ultimately, Coen is on the mark in saying that the science of earthquakes needs a strong human element. The Kantian ideal of earthquake studies—expressed in *The Earthquake Observers*, by a quote from Kuno Fischer, as “focused squarely on the lawful necessity of nature”—is a useful approach but incomplete without considering the effects of earthquakes on people. The public, after all, is at risk from earthquakes, and there is no substitute for public participation in comprehending and mitigating that risk.

References

1. <http://earthquake.usgs.gov/research/dyfi>.
2. D. J. Wald, V. Quitoriano, B. Worden, M. Hopper, J. W. Dewey, *Ann. Geophys.* **54**, 688 (2011).
3. E. S. Cochran *et al.*, *Ann. Geophys.* **54**, 728 (2011).
4. G. Hartwig, *Volcanoes and Earthquakes: A Popular Description of the Movements in the Earth's Crust* (Longman, Green, London, 1887).

10.1126/science.1235758

SCIENCE FESTIVALS

Serpentine Figures in Edinburgh

Deborah Dixon and Elizabeth Straughan

This year marks the 25th anniversary of the Edinburgh International Science Festival, an event that showcases the past, present, and possible future of scientific discovery and, in particular, the many contributions of Enlightenment Scotland. Drawing on archives and expertise from a wealth of sites across the city (from the University of Edinburgh to the National Botanic Garden) and using a series of atmospheric spaces (from St. Giles Cathedral to St. Andrews Square), the festival emphasizes not so much the role of science in culture as science as culture.

The festival accomplishes this understanding of science as culture in diverse ways. It is an engine for the public communication of science, accomplished through learning activities, talks, debates, and exhibi-



Jason Hackenwerth's *Pisces* (2013).

tions. Key participants in these endeavors are the blue-t-shirted science communicators, who explain how science underpins all manner of everyday activities and objects, from cooking to plastic bags. The festival also displays a sustained emphasis on what it is like to practice science, most evident in the hands-on activities on offer at the City Art Centre, from unwrapping mummies to building robots. In addition, it includes a series of events that explicitly seek to break down the notion of a distinction between the sciences and the arts—by referencing a shared sense of curiosity and wonder, certainly, but also by illustrating how ideas and concepts from both traditions have been and are being translated, reworked, and reconfigured.

Two such translational events, in which scientific ideas and concepts become manifest in an artistic medium, have been introduced into the Grand Gallery of the National Museum of Scotland—a three-story, glass-and-cast-metal concourse with wraparound balconies that formerly was the foyer to the Edinburgh Museum of Science and Art (1866–1904). Artist Jason Hackenwerth's *Pisces* (2013) is a site-specific latex and air sculpture that was assembled from balloons in the Grand Gallery and then hoisted up and hung from the ceiling. The piece draws on Hackenwerth's prior work, wherein the balloon scaffolding and tumescent forms reference marine and insectoid organisms that loom large above an audience yet retain an airy, playful quality. In addition, *Pisces* very much responds to the content and form of the Gallery space, as its tightly wound curves replicate what mathematician Jacob Bernoulli called the *Spira mirabilis* (marvellous spiral), displayed in the

shell collection on the museum's third floor. The undulating surfaces of *Pisces* also reflect the rounded, Romanesque Revival arches of the Gallery's roof and supports.

Curves, and their reiteration, are also central to *Chaos and Contingency* (2013), a performance by the Janis Claxton Dance Company, with a cast of nine dancers from Scotland and China, and music composed by Philip Pinsky. Although the dance is performed on the ground floor, it has been designed with the Grand Gallery's architecture of vision in mind, as each balcony affords a differing view of the clustering and dispersal of bodies. At the outset of the piece, the dancers slowly spread out with a synchronization that sees them gently lift their arms, twist their bodies, and raise their heads skyward. Such mirroring does not last, however, as individually and in groups the dancers introduce small variations in both pose and pace; these in turn are mirrored and varied as the dance proceeds. There is an animal physicality to how the bodies swirl, roll, swerve, and almost col-

lide. It is this fleshiness that suggests a tension between a disciplining of the body (and of the collective) and a continual evolution of form.

Both *Pisces* and *Chaos and Contingency* can be considered examples of what art historians call the *figura*

serpentinata (serpentine figure). In them, swirling convex and concave shapes blur the boundaries between organisms, unifying those into a new body that challenges prior taxonomies but that also finds a balancing point wherein each element occupies a new resting place. This serpentine figure finds resonance in various scientific concepts and models, from the double helix of DNA to the spiraling arms of galaxies. Yet, as the architectural theorist, landscape designer, and artist Charles Jencks reminded the audience at his 23 March lecture, “Landforms,” art and science are not themselves in an equal dialogue. Where science holds the upper hand in regard to content—that is, in its exquisite and highly specialized descriptions of how the universe works—art retains the power of the spectacle. Although metaphors such as the serpentine figure provide something of a common ground between art and science, there is no resolution. Nor is there a point around which the two can be counterbalanced. Perhaps it is this irresolution and falling apart—this very asymmetry—that animates such interdisciplinary efforts?

10.1126/science.1238516

The reviewers are at the School of Geographical and Earth Sciences, University of Glasgow, East Quadrangle, University Avenue, Glasgow G12 8QQ, Scotland, UK. E-mail: deborah.dixon@glasgow.ac.uk (D.D.); libby.straughan@glasgow.ac.uk (E.S.).

SCIENCE EDUCATION

Opportunities and Challenges in Next Generation Standards

E. K. Stage,^{1*} H. Asturias,¹ T. Cheuk,² P. A. Daro,³ S. B. Hampton³

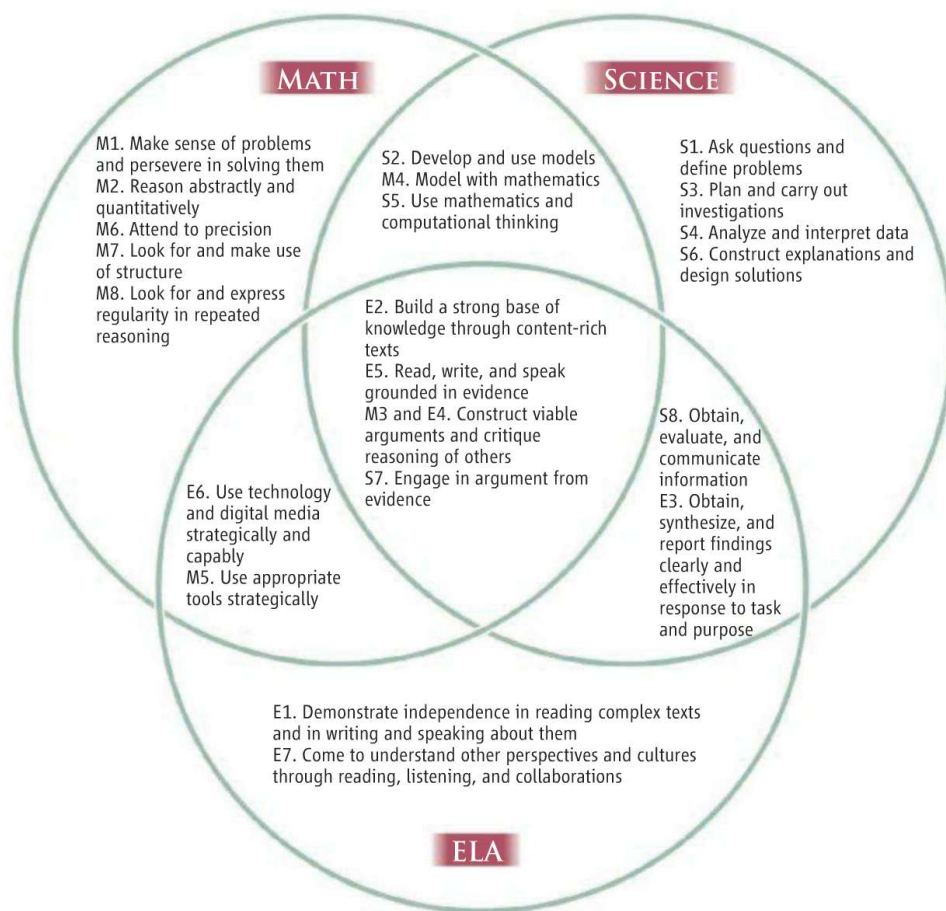
Imagine that politicians and the people they represent understood how human activity impacts Earth, including climate. And imagine that they had learned how to evaluate claims, argue from evidence, and understand models. These understandings and practices are prominent in the U.S. National Research Council (NRC) framework to guide the next iteration of standards for U.S. elementary and secondary school students (1). We discuss how aspects such as authorship, coordination among subject areas, and broader goals of college and career readiness give reason to believe that this effort will be more successful than previous attempts to use standards to improve science education (2).

Concurrent development in English Language Arts (ELA) (“literacy”) and Mathematics, under the Common Core State Standards (CCSS) (3, 4), has provided the opportunity to build on the strengths of these literacy and math documents from a science education perspective. Closely following the CCSS, the Next Generation Science Standards (NGSS) are being developed by Achieve, a nonprofit organization, working directly with 26 lead states (5). This structure acknowledges that the standards will be adopted and implemented at the state level.

Past educational standards were developed by professional organizations on behalf of scientists and educators and in different subject areas independently, yielding more material than any K–12 school system (kindergarten to high school) could teach well (6, 7). Now there is a call for “fewer, clearer, and higher” standards (8).

Building on Literacy and Math

The CCSS focus not only on what it will take to become a successful student in higher education but also a successful employee. Broadening the scope in this way, skills and abilities that support civic participation are explicit in the standards. Reading standards



Relations and convergences in literacy (3), math (4), and science and engineering (1) practices. Adapted from (12).

give earlier and more extensive treatment of informational text than in the past. This is echoed in the writing standards; “The ability to write logical arguments based on substantive claims, sound reasoning, and relevant evidence is a cornerstone” (9). Writing standards include in-depth research with an emphasis on analysis and presentation. Standards for speaking and listening include “Integrate multiple sources of information presented in diverse formats and media (e.g., visually, quantitatively, orally) in order to make informed decisions and solve problems, evaluating the credibility and accuracy of each source and noting any discrepancies among the data” (3).

We see a similar emphasis on reasoning and problem-solving in the math standards.

Comparisons with high-performing countries find that spending more time on fewer topics gets better results. Thus, the math standards emphasize focus and coherence rather than covering topics in a curriculum that is a “mile wide and an inch deep” (10). Greater depth in each topic comes from students’ development of mathematical expertise defined by eight standards for mathematical practice.

The math standards take an overdue step toward greater synergy with science by introducing modeling in secondary grades. The math standards define modeling as “the process of choosing and using appropriate mathematics and statistics to analyze empirical situations, to understand them better, and to improve decisions” (4). The elaboration of the basic modeling cycle resonates with the

¹Lawrence Hall of Science, University of California, Berkeley, Berkeley, CA 94720, USA. ²Graduate School of Education, Stanford University, Stanford, CA 94305, USA. ³National Center on Education and the Economy, Washington, DC 20006, USA.

*Corresponding author. stage@berkeley.edu

writing standards and with the science practices, e.g., “(5) validating the conclusions by comparing them with the situation, and then either improving the model or, if it is acceptable, (6) reporting on the conclusions and the reasoning behind them. Choices, assumptions, and approximations are present throughout this cycle” (4).

Literacy and math standards include practices that are challenging to teach in science without support from teachers of other subjects. Standards for Speaking and Listening include, “Evaluate a speaker’s point of view, reasoning, and use of evidence and rhetoric” (3). Standards for Mathematical Practice include, “Construct viable arguments and critique the reasoning of others” (4).

Operationalizing Inquiry

In this promising context, science standards have been drafted, working from the NRC framework, that operationalized “inquiry” with eight practices of science and engineering: (i) asking questions and defining problems; (ii) developing and using models; (iii) planning and carrying out investigations; (iv) analyzing and interpreting data; (v) using mathematics and computational thinking; (vi) constructing explanations and designing solutions; (vii) engaging in argument from evidence; and (viii) obtaining, evaluating, and communicating information (2).

The framework attempted to narrow the number of core disciplinary ideas, although reviewers of draft science standards have said that the volume of content undermines the sense making required by the practices (11). The framework retained the idea of crosscutting concepts (e.g., structure and function, stability and change of systems), and argued that practices, core disciplinary ideas, and crosscutting concepts should not be taught or assessed separately from each other. Each draft science performance expectation incorporates one or more disciplinary idea, practice, and/or crosscutting concept. These performance expectations also cross-reference the literacy and math standards; the convergence is shown in the chart (12).

Science educators have decried the common practice of reading textbooks instead of doing investigations; the former is still alive and well (13). Literacy educators are concerned about increased emphasis on informational text in the CCSS (14). It is time to embrace the coherence and learning that can be achieved by making meaningful connections between and among direct experience with science and engineering practices and reading, writing, speaking, and listening (15).

What’s Next?

Forty-five states have adopted the CCSS. If a substantial number of states adopt the NGSS, it increases the likelihood that developers and publishers of instructional and assessment materials will focus on creating a common set of tools, at least at elementary and middle grades. If colleges and universities accept high school courses that are based on the standards and the College Board continues to revise the Advanced Placement syllabi, high schools are more likely to follow them.

In addition to sufficient time and resources for educators and parents to learn how to support these more ambitious expectations, there are several challenges that scientists, educators, and policy-makers should consider. Advocates for high-quality science education for all students need to participate in conversations at the local and state level where educational policy is enacted. Scientists from higher education, research organizations, and corporations influence science education and can align their contributions with educational goals in the standards.

Historically, the United States has provided limited opportunity to learn science to most of its students and advanced training to a privileged few, focusing on the pipeline for future scientists and innovators without concomitant attention to a science literacy for citizenship. The system needs to be transformed to affirm high standards of accomplishment for all students and to provide resources for all students to reach them (8).

Although the literacy and math standards were widely adopted, and 26 states have served as partners in developing NGSS, momentum may be slowing; some states may reject the NGSS because of the inclusion of evolution and climate change (16). The National Center for Science Education, a defender of teaching evolution for more than three decades, broadened its mission to include the defense of teaching climate science.

Science education benefits from the learning sciences; scientists interested in the most effective teaching of science need to learn from education research. Formal schooling has been criticized as ineffective at motivating and inspiring students (17) and inadequate at recognizing the relation between interest and accomplishment (18). The NGSS can provide a platform for formal education to become more motivating. Many people are inspired by science in informal settings; parallel attention to the NGSS can contribute to “a wide-ranging and thriving ecosystem of opportunities that respond to the needs of children as well as commu-

nities” (19). Education and public outreach activities associated with research grants, whether in or out of school, should provide both preparation and inspiration. Local school districts, after-school providers, and informal science institutions need to create a coherent strategy for the regional science learning ecosystem.

This new round of standards development is an opportunity to improve science education that comes around once for each generation. We need to inform ourselves, figure out whether and how we want to get involved, and be intentional about our participation.

References

1. Board on Science Education, National Research Council (NRC), *A Framework for K-12 Science Education: Practices, Crosscutting Concepts, and Core Ideas* (National Academies Press, Washington, DC, 2011).
2. National Academy of Engineering and Committee on Standards for K-12 Engineering Education, NRC, *K-12 Standards for Engineering?* (National Academies Press, Washington, DC, 2010).
3. Center for Best Practices, National Governors Association (NGA), and Council of Chief State School Officers, *Common Core State Standards for English Language Arts* (NGA, Washington, DC, 2010); www.corestandards.org/ELA-Literacy.
4. Center for Best Practices, NGA, and Council of Chief State School Officers, *Common Core State Standards for Mathematics* (NGA, Washington, DC, 2010); www.corestandards.org/Math.
5. Next Generation Science Standards, www.nextgenscience.org/next-generation-science-standards.
6. American Association for the Advancement of Science, *Project 2061, Benchmarks for Science Literacy* (Oxford Univ. Press, New York, 1993).
7. National Committee for Science Education Standards and Assessment, NRC, *National Science Education Standards* (National Academies Press, Washington, DC, 1996).
8. Commission on Mathematics and Science Education, *The Opportunity Equation: Transforming Mathematics and Science Education for Citizenship and the Global Economy* (Carnegie Corporation of New York, New York, 2009).
9. Common Core, www.corestandards.org/about-the-standards/key-points-in-english-language-arts.
10. W. H. Schmidt, C. C. McKnight, S. A. Raizen, *A Splintered Vision: An Investigation of U.S. Science and Mathematics Education* (Kluwer Academic Publishers, Boston, MA, 1997).
11. J. Coffey, B. Alberts, *Science* **339**, 489 (2013).
12. T. Cheuk, Comparison of the three content standards: CCSS-ELA, CCSS-Mathematics, and NGSS (2012); <http://ell.stanford.edu/content/science>.
13. E. R. Banilower et al., *Report of the 2012 National Survey of Science and Mathematics Education* (Horizon Research, Chapel Hill, NC, 2013).
14. C. Gewertz, *Educ. Week* **32**(12), S2 (2012).
15. P. D. Pearson, E. Moje, C. Greenleaf, *Science* **328**, 459 (2010).
16. E. W. Robelen, *Educ. Week* **32**(19), 13 (2013).
17. C. Weiman, *Issues Sci. Technol.* **2012** (Fall) (2012).
18. R. H. Tai, C. Qi Liu, A. V. Maltese, X. Fan, *Science* **312**, 1143 (2006).
19. President’s Council of Advisors on Science and Technology, *Prepare and Inspire: K-12 Education in Science, Technology, Engineering, and Math (STEM) for America’s Future* (Office of the President, Washington, DC, 2010).

SCIENCE EDUCATION

Driven by Diversity

Joan Ferrini-Mundy

Diversity of perspectives, ideas, and priorities comes from varied backgrounds, experiences, and cultures, and can help shape science and engineering. As science increasingly is done in teams, collaborations bring diversity to research. Diverse experiences and interactions can support cognitive growth and critical thinking (1), can benefit problem-solving (2), and are priorities among STEM (science, technology, engineering, and math) businesses for global competition (3).

Currently, U.S. STEM education efforts are not fully drawing upon the full scientific potential of all demographic groups and populations. Hispanics, African Americans, and American Indians and/or Alaska natives made up 26% of the general population, but only 9% of STEM workers, and 11% of STEM degree holders in 2008 (4). Without change and a strong inclusion culture to increase the representation of women and minorities, the STEM workforce will not reflect the broader U.S. population, posing a challenge to economic efficiency and social equity (5). We must embrace and encourage more comprehensive and strategic approaches in order to advance diversity and inclusion in the STEM workforce and beyond.

How to Provide the STEM Workforce for Tomorrow?

Equitable opportunity to learn from excellent K–12 teachers. The distribution of STEM teacher expertise in high-needs schools from kindergarten to high school (K–12) is an issue. As of 2012, 69% of science teachers in low-poverty schools had advanced degrees versus 49% in high-poverty schools (4). Research is needed to better understand how teacher background, knowledge, and preparation relate to effective teaching. Advanced secondary school courses, such as physics and calculus, are disproportionately offered in schools with lowest Black and Hispanic enrollments (6). Unless all youth in all schools have the opportunity to learn significant STEM in their early years, it seems improbable that students will be prepared,

either in adequate numbers or with appropriate knowledge of content and practices, to make choices leading to successful STEM careers. State-led efforts to implement Common Core State Standards and Next Generation Science Standards have potential to lead to common opportunity to learn for all students.

Widespread opportunity to engage in authentic, inspiring STEM learning inside and outside of school. Young children's thinking about science is "sophisticated"; they can understand and reason about complex STEM concepts (7). All children should have opportunities to be engaged in challenging STEM learning activities. Students who have the chance to participate in authentic experiences that model the excitement and rewards of STEM have a good start toward entering STEM pathways (8). Outside-of-school or "informal" STEM learning environments such as museums, science centers, and media settings can "make science accessible, meaningful, and relevant for diverse students by connecting their home and community cultures to science" (9). Students who are able to develop strong science identities and self-efficacy are more likely to persist in STEM (10); continued attention to developing these qualities in all learners is warranted.

Recruit and retain a wide diversity of students into undergraduate STEM courses and majors. Women and minorities make up about 70% of college students but only receive about 45% of STEM degrees (11). Recommendations to retain undergraduate STEM majors include catalyzing the adoption of evidence-based teaching practices and addressing the mathematics preparation gap (11). College students are less likely to leave a STEM discipline if they have had the opportunity to engage in research with a professor, with strongest impact on African American students and second-year undergraduates (12).

Preparation for all graduate students for the international science workplace. The nature and practice of science and collaboration are changing more rapidly than ever. The number of articles with authors from multiple institutions and countries has increased (4). International collaboration is already a norm for scientists. Stronger career preparation is needed, with professional

Evidence is growing on benefits, and approaches, to increase diversity in the science and engineering workforce.

development in innovation, communication, and enhanced collaborations with industry (13, 14), and international experiences when possible.

Vannevar Bush (15), writing about increasing the scientific capital of basic research, said "First, we must have plenty of men and women trained in science, for upon them depends both the creation of new knowledge and its application to practical purposes." The National Science Foundation remains committed to broadening participation because it is essential for the nation's scientific progress.

References and Notes

1. C. Loes et al., Effects of diversity experiences on critical thinking skills over four years of college; www.education.uiowa.edu/centers/docs/cdre-documents/Loes_Pascarella_and_Umbach_2012_3.pdf?sfvrsn=0.
2. S. E. Page, *The Difference: How the Power of Diversity Creates Better Groups, Firms, Schools, and Societies* (Princeton Univ. Press, Princeton, NJ, 2007).
3. Bayer Corp., *STEM Education, Science Literacy and the Innovation Workforce in America: 2012 Analysis and Insights from the Bayer Facts of Science Education Surveys 1995–2011* (Bayer Corp., Pittsburgh, PA, 2012).
4. National Science Board, *Science and Engineering Indicators 2012* (NSB, Arlington, VA, 2012).
5. A. P. Carnevale et al., *STEM: Science, Technology, Engineering, Mathematics* (Georgetown University, Washington, DC, 2011).
6. U.S. Department of Education, Civil Rights Data Collection; <http://ocrdata.ed.gov/>.
7. R. A. Duschl et al., Eds., *Taking Science to School: Learning and Teaching Science in Grades K–8* (National Academies Press, Washington, DC, 2007).
8. R. F. Subotnik, R. H. Tai, J. Almarode, paper presented at the NRC Workshop on Successful STEM Education in K–12 Schools, Washington, DC 10–11 May 2011; http://sites.nationalacademies.org/DBASse/BOSE/DBASse_071087#.UWQVM1fwjXN.
9. National Academy of Sciences, National Academy of Engineering, Institute of Medicine, *Expanding Underrepresented Minority Participation: America's Science and Technology Talent at the Crossroads* (National Academies Press, Washington, DC, 2011).
10. Z. Hazari et al., *J. Res. Sci. Teach.* **47**, 978 (2010).
11. President's Council of Advisors on Science and Technology, *Engage to excel: Producing one-million additional college graduates with degrees in science, technology, engineering, and mathematics* (Office of the President, Washington, DC, 2012).
12. S. R. Gregerman et al., *Rev. Higher Educ.* **22**(1), 55 (1998).
13. Biomedical Research Workforce, Working group report (National Institutes of Health, Bethesda, MD, 2012).
14. Council of Graduate Schools and Educational Testing Service, *Pathways Through Graduate School and into Careers* (CGS and ETS, Washington, DC, and Princeton, NJ, 2012).
15. V. Bush, *Science, the Endless Frontier* (Government Printing Office, Washington, DC, 1945).

Acknowledgment: Special thanks to H. Lakhani, Education and Human Resources directorate, National Science Foundation.

10.1126/science.1235521

Assistant Director for Education and Human Resources, National Science Foundation, Arlington, VA 22230, USA.

E-mail: jferrini@nsf.gov

NEUROSCIENCE

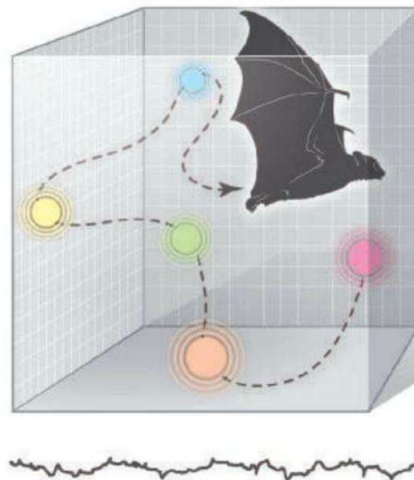
3D Mapping in the Brain

Caswell Barry^{1,2,3} and Christian F. Doeller⁴

What's it like to be a bat? If a rat could fly and echolocate, would it navigate and determine its position in the same way? In other words, could the rat truly understand three-dimensional (3D) space? Or is a bat's brain functionally specialized for life in the air? On pages 367 and 363 of this issue, Yartsev *et al.* (1) and Heys *et al.* (2) examine the neural encoding of 3D space and basic neuronal processing in bats and discover fundamental differences in the way species represent their own location.

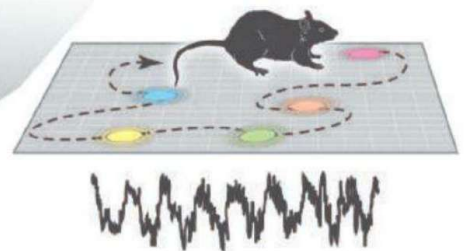
In the mammalian brain, the ability to remember and navigate through space has been linked to the hippocampus. The best evidence for this association lies with hippocampal neurons called "place cells," which represent an animal's position (3). However, ground-dwelling rodents have provided the dominant model for such spatial studies, leaving open the question of whether the hippocampal representation of space truly extends into 3D. Furthermore, it is also unknown if the strong theta-band (6 to 12 Hz) modulation of hippocampal electrical activity (4), which is thought to contribute to the spatial response of place cells in rodents (5), plays a similar role in other animals. By describing hippocampal electrical activity in flying bats and in isolated bat medial entorhinal cortical tissue (the entorhinal cortex is a major cortical input to the hippocampus), Yartsev *et al.* and Heys *et al.* find that bats exhibit 3D "place fields," without any apparent theta-band modulation in the hippocampus or in the membrane potential resonance of specific entorhinal inputs to the hippocampus (see the figure).

Place cells have been characterized in animals as phylogenetically diverse as rats (3), humans (6), and crawling bats (which can move slowly on all fours) (7). Place cells represent an animal's location with spatially selective patterns of electrical activity—each cell "firing" only when the animal is in a specific position, its place field. Typically, place fields are stable and distributed across the environment. A population of place cells



Bats and rats use different neural maps to navigate in their natural spatial surroundings.

Maps in rats and bats. (Right) As a rat is navigating in 2D space, there is strong electrical activity in hippocampal place cells and grid cells in the medial entorhinal cortex accompanied by theta-band oscillations. **(Left)** As a bat navigates in 3D, there is low-frequency and lower-power oscillations in these neurons. Color patches indicate 2D and 3D place fields in space where neurons signal the animal's position, providing either a 2D or a 3D map of the animal's world.



thus provides a map-like representation of the animal's world (3). This representation is impressively flexible—a new map is quickly generated in a new environment. Attempts with rodents to determine if place cell activity is modulated in 3D could not truly explore volumes of space. Rats trained to climb on a vertical peg board and staircase showed that place cell firing was weakly modulated in the vertical dimension (8), but it was unclear if this constituted a real 3D space code. Yartsev *et al.* tackled this question by recording from fruit bats as they flew around a large cage—a technically impressive feat requiring wireless, light-weight recording equipment and multiple tracking cameras. The authors demonstrate that the hippocampal spatial code is indeed 3D—the bats had place fields that were confined to specific, evenly distributed volumes of the cage with no tendency to be compressed in any one dimension. Although the 3D place fields were stable for several hours, it is unclear if they exhibit the long-term stability of 2D fields and if they can rapidly form new representations when the animals enter a new space.

Does the bat hippocampus use the same mechanisms to construct space as the rodent hippocampus? A key functional difference revealed by Yartsev *et al.* is that bat place cells exhibit no theta-band modulation of their spike trains—in rodents, this rhythmic modulation of neuronal firing is evident whenever the animals are in motion (4). Indeed, several computational models describe the spatial activity of grid cells (9), an input to the

hippocampus, in terms of theta-band oscillators (5). Grid cells are neurons in the medial entorhinal cortex, and like place cells, exhibit stable spatially constrained firing, but tessellate the environment with an array of regularly distributed firing fields. Again, grid cells appear to be a mammalian phenomenon—they are found in rodents (9), and similar spatial representations exist in humans (10). The spatial grid array can be described as an interference pattern occurring between theta-band oscillations in grid cells or their inputs, the frequencies of which track the animal's velocity (5). Such a configuration generates regular grid cell firing and provides a mechanism by which information about self-motion—speed and direction—can update a representation of location, a process known as path integration.

An absence of theta-band modulation of bat grid cells had been reported (11); however, the results were debated because the cells of the slowly crawling bats emitted too few spikes for a conclusive analysis (12). To address this, Heys *et al.* directly examined the membrane dynamics of bat medial entorhinal stellate cells in brain slices. In rodents, these neurons show subthreshold membrane potential resonance and oscillate at theta-band frequency—good evidence for interference models. However, the authors found no theta-band resonance in the bat stellate cells. It is therefore unlikely that the spatial firing of bat grid cells is produced by theta-band membrane potential resonance in medial entorhi-

¹Institute of Cognitive Neuroscience, ²Institute of Neurology, and ³Institute of Behavioural Neuroscience, University College London, UK. ⁴Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, Netherlands. E-mail: caswell.barry@ucl.ac.uk; christian.doeller@donders.ru.nl

nal cortex—as envisaged in one formulation of the interference model (13).

Different implementations of the interference model have placed the velocity-controlled oscillators in other brain regions, such as the medial septum (14), and do not necessarily require membrane potential oscillations to be present in the entorhinal cortex (some degree of theta-band modulation of grid cell firing would be expected). Heys *et al.* did observe membrane potential oscillations of medial entorhinal cells, but at frequencies below the normal theta range (1 to 2 Hz), raising the possibility that oscillatory interference might be occurring at lower frequencies. These findings may be indicative of differences in the nature of neuronal oscillations between species. For instance, hippocampal theta-band oscillations in humans have a much lower frequency than in rats (15). Alternatively, a shift to lower-frequency

oscillations might avoid unwanted interactions with the processing of bat echolocation epochs which occur at 6 to 12 Hz. Indeed, at least one species of bat shows theta-like modulation in hippocampal neurons when echolocating (7).

It may be that the adaptations that allow bats to function in, and encode, 3D space have dramatically altered the way they path integrate—as such, their grid cells may be functionally different from those in rodents and humans. Characterizing grid cells in flying bats will help to answer some of these questions.

References and Notes

1. M. M. Yartsev, N. Ulanovsky, *Science* **340**, 367 (2013).
2. J. G. Heys, K. M. MacLeod, C. F. Moss, M. E. Hasselmo, *Science* **340**, 363 (2013).
3. J. O'Keefe, L. Nadel, *The Hippocampus as a Cognitive Map*. (Oxford Univ. Press, ed. 1, 1978).
4. G. Buzsáki, *Neuron* **33**, 325 (2002).
5. N. Burgess, C. Barry, J. O'Keefe, *Hippocampus* **17**, 801 (2007).
6. A. D. Ekstrom *et al.*, *Nature* **425**, 184 (2003).
7. N. Ulanovsky, C. F. Moss, *Nat. Neurosci.* **10**, 224 (2007).
8. R. Hayman, M. A. Verriotti, A. Jovalekic, A. A. Fenton, K. J. Jeffery, *Nat. Neurosci.* **14**, 1182 (2011).
9. T. Hafting, M. Fyhn, S. Molden, M.-B. Moser, E. I. Moser, *Nature* **436**, 801 (2005).
10. C. F. Doeller, C. Barry, N. Burgess, *Nature* **463**, 657 (2010).
11. M. M. Yartsev, M. P. Witter, N. Ulanovsky, *Nature* **479**, 103 (2011).
12. C. Barry, D. Bush, J. O'Keefe, N. Burgess, *Nature* **488**, E1 (2012).
13. L. M. Giocomo, E. A. Zilli, E. Fransén, M. E. Hasselmo, *Science* **315**, 1719 (2007).
14. A. C. Welday, I. G. Shlifer, M. L. Bloom, K. Zhang, H. T. Blair, *J. Neurosci.* **31**, 16157 (2011).
15. R. Kaplan *et al.*, *PLoS Biol.* **10**, e1001267 (2012).

Acknowledgments: C.B. is supported by a University College London Excellence Fellowship and a Wellcome Trust Principal Fellowship (awarded to N. Burgess). C.F.D. is supported by the European Research Council (ERC-StG RECONTEXT).

10.1126/science.1237569

CLIMATE CHANGE

Climate's Dark Forcings

Meinrat O. Andreae^{1,2} and V. Ramanathan^{2,3}

The black soot coming out of the tailpipes of diesel trucks is a nuisance familiar to every highway traveler. Soot also endangers the health of untold numbers of women and their families exposed to smoke from traditional cookstoves burning biofuels and coal. But in addition to irritating our noses and lungs, this pollutant, also known as black carbon (BC), is the strongest absorber of solar radiation in the atmosphere. The magnitude of global warming from BC, as well as its regional effects, has been the subject of intense debate. In a recent comprehensive assessment, Bond *et al.* (1) have synthesized available model results and observations, and propose a “best estimate” for BC's global climate forcing. Their estimate is almost twice as high as values commonly discussed (2). What causes such large discrepancies between estimates, and what are the implications for the global and regional climate effects of BC?

Unlike greenhouse gases, BC is not a single, chemically defined substance with constant physical properties. In addition to the aggregates of nanometer-scale carbon spher-

ules traditionally thought of as BC, the atmosphere contains light-absorbing organic or “brown” carbon (BrC) (3). BrC may account for 15 to 50% of light absorption in the atmosphere and in snow and ice (1, 4, 5) and has different optical properties and source and sink patterns from BC. In addition to combustion sources, especially biomass burning, BrC is also produced by atmospheric chemical reactions, a source not considered in emission inventories.

BrC is sometimes included implicitly in climate models constrained by BC measurements, because different BC measurement techniques may include some or all BrC. However, most models have ignored BrC absorption and, as a result, concluded that the combination of BC and nonabsorbing organic carbon leads to net cooling. This has been challenged by two recent studies (5, 6). It is essential to improve measurement techniques for BrC and to include it explicitly in models.

BC (including BrC) influences climate through numerous mechanisms. In addition to causing atmospheric heating and surface dimming, BC-containing aerosols affect cloud optical properties and precipitation behavior. This in turn affects the energy budget of the atmosphere (7). The global net BC forcing is obtained by integrating over

Uncertainties about the properties and amounts of atmospheric black carbon complicate efforts to understand its regional and global effects on climate.

all mechanisms. Because most earlier studies have included only a subset of mechanisms, one must be very careful when making comparisons.

Bond *et al.*'s “all mechanisms” forcing estimate of +1.1 W m⁻² (with a large uncertainty) is about twice as high as that of UNEP/WMO (2), mostly because of higher values for the absorption by BC in the atmosphere. Yet, their estimate of 0.88 W m⁻² for the forcing from light absorption by present-day BC is almost identical to that from a previous study (0.9 W m⁻²) (8). This agreement is instructive, because the two studies use atmospheric models, but are otherwise based on very different approaches.

Ramanathan and Carmichael's estimate (8) is based on satellite and ground-based light absorption data from the AERONET network of more than 140 sites around the world. In contrast, Bond *et al.* derive absorption estimates from emission inventories and atmospheric models. They initially obtain values lower than supported by observations; only when they scale up their results to agree with the AERONET data do the two studies converge. This suggests that underestimation of atmospheric BC absorption by as much as a factor of three is the primary culprit for the lower forcing estimates in most earlier models. Either the models are missing a major BC

¹Biogeochemistry Department, Max Planck Institute for Chemistry, 55020 Mainz, Germany. ²Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093, USA. ³TERI Institute, New Delhi 110 070, India. E-mail: m.andreae@mpic.de

source or the emission inventory values are not applicable worldwide.

Summing up all forcing terms may be appropriate for a global estimate, but the relative importance of the individual effects differs between regions, especially when considering the effects on precipitation. Furthermore, the regional net forcing from a source such as biomass burning can even change sign from region to region (see the figure). Climate change will be more sharply felt at the regional scale and through changes in water availability than through an increase in average global temperature.

Because BC is concentrated near its sources, it introduces spatial gradients in solar heating of the atmosphere and surface. Over South Asia, this differential dimming of the surface weakens the north-south gradient in the solar heating of the Indian Ocean and the land-ocean contrast in surface solar heating. The net effect is a weakened monsoon circulation and a reduction in evaporation, reducing the amount of water available for rainfall (8).

These coupled ocean-atmosphere processes act on seasonal time scales. On shorter time scales, BC warms the troposphere above 3 km, intensifying monsoon circulation (9) and modulating intraseasonal monsoon variations (10). Furthermore, the aerosol particles that contain BC reduce precipitation efficiency in small clouds but enhance it in deep clouds and make the clouds grow taller (7). The resulting vertical redistribution of heat affects monsoon circulation and tropical precipitation.

The combined radiative, microphysical, and dynamical effects of absorbing aerosols may explain observed changes since the 1950s in the Indian and East Asian Monsoon and the Sahelian drought (1, 8, 9). On an even larger scale, model results suggest that BC is to a great extent responsible for the observed expansion of the tropical belt and the poleward shift of the tropical jet and extratropical storm tracks (11). In Earth's cold regions, heating of the air and darkening of snow by BC may accelerate Arctic sea ice retreat and melting of Himalayan glaciers (1, 12). If, as concluded by Bond *et al.*, BC absorption has been strongly underestimated in previous models, the regional effects may be much larger than models have assumed. A reexamination is urgently required.

Like all climate change assessments, the conclusions of Bond *et al.* are based mainly on models. However, the representation of aerosol and cloud processes in climate models has severe shortcomings. The increasing complexity of recent model versions does



Smoke properties and cloud interactions. (A) The smoke from the 12 August 2009 fire (white box) northwest of Santa Cruz, California, is light colored because it burns in moist vegetation. Interaction of the smoke plume with clouds off the Monterey Peninsula (center) increases the reflectance of the clouds. (B) The smoke from a fire in extremely dry vegetation in the Great Victoria Desert, Australia (near 29°S, 129°E), on 17 January 2013 is nearly black. A bright cumulus cloud rises from the top of the plume.

not necessarily imply improved model skill. Better observational constraints are needed at all scales, from aerosol microphysics to global satellite studies of cloud properties.

Is BC mitigation, as suggested by UNEP/WMO, worth the effort, given the large uncertainties in BC forcings? Most BC sources coemit other species, such as organic particles and SO₂, which have a negative forcing. The forcings from BC and these other species nearly cancel each other in present models (1), mainly because of the large cooling effect of open biomass burning. However, BC from diesel and coal burning may have a substantial net warming effect despite cooling from coemitted species (13). In California, BC concentrations fell by 50% from 1988 to 2008, largely as a result of diesel emission reductions (14), whereas coemitted species showed no trends. Mitigation could thus target diesel exhaust, as well as traditional cookstoves that burn biofuels.

The potentially large effects on regional precipitation and circulation and on glacier melting offer further incentives to mitigate BC. However, the strongest motivation may come from the expected health benefits. An estimated 3.5 million deaths annually are attributed to household air pollution from solid fuels and an additional 3.1 million deaths to ambient particles, including BC (15). Finally, the discussion about BC and other short-lived climate forcers must not obscure the 38-billion-ton and grow-

ing gorilla in the room—the amount of CO₂ emitted annually into the atmosphere—which needs to be cut sharply and promptly to avoid accelerating climate change.

References and Notes

1. T. C. Bond *et al.*, *J. Geophys. Res.* **10.1002/jgrd.50171** (2013).
2. UNEP/WMO, *Integrated Assessment of Black Carbon and Tropospheric Ozone* (United Nations Environment Programme, Nairobi, Kenya, and World Meteorological Organization, Geneva, Switzerland, 2011).
3. M. O. Andreae, A. Gelencsér, *Atmos. Chem. Phys.* **6**, 3131 (2006).
4. T. W. Kirchstetter, T. L. Thatcher, *Atmos. Chem. Phys.* **12**, 6067 (2012).
5. C. E. Chung, V. Ramanathan, D. Decremier, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 11624 (2012).
6. R. Bahadur, P. S. Praveen, Y. Y. Xu, V. Ramanathan, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 17366 (2012).
7. D. Rosenfeld *et al.*, *Science* **321**, 1309 (2008).
8. V. Ramanathan, G. Carmichael, *Nat. Geosci.* **1**, 221 (2008).
9. W. K. M. Lau, K.-M. Kim, *Geophys. Res. Lett.* **37**, L16705 (2010).
10. M. G. Manoj, P. C. S. Devara, P. D. Safai, B. N. Goswami, *Clim. Dyn.* **37**, 2181 (2011).
11. R. J. Allen, S. C. Sherwood, J. R. Norris, C. S. Zender, *Nature* **485**, 350 (2012).
12. B. Q. Xu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 22114 (2009).
13. M. Z. Jacobson, X.-M. Shao, R. Holzworth, *J. Geophys. Res.* **115**, D14209 (2010).
14. R. Bahadur, Y. Feng, L. M. Russell, V. Ramanathan, *Atmos. Environ.* **45**, 1162 (2011).
15. S. S. Lim *et al.*, *Lancet* **380**, 2224 (2012).

Acknowledgments: M.O.A. acknowledges the Max Planck Society and V.R. the National Science Foundation. We thank T. C. Bond and D. W. Fahey for thoughtful discussions and L. Ellison for help with the figure.

10.1126/science.1235731

Heterochronic Genes Turn Back the Clock in Old Neurons

Paola Nix and Michael Bastiani

Although some neuron types regenerate better than others, all neurons lose the ability to regenerate with age. This intrinsic decline is the primary cause of regeneration failure even in permissive environments. This was shown in 1995 by comparing the regeneration ability of retinal neurons from different aged retinas growing into tectums of different ages (1). Embryonic retinal axons regrew into tectum of any age, including older tectum with an inhibitory glial environment, whereas postnatal day 2 or older retinal axons failed to regrow even into embryonic tectum. This indicated a “programmed” loss of axon regeneration ability with neuron age. Similarly, young hindbrain neurons transplanted into older spinal cords could regenerate axons into a normally inhibitory myelinated environment (2). Despite the clear therapeutic implications of these observations, the underlying molecular mechanisms controlling age-dependent regenerative capacity were unclear. On page 372 in this issue, Zou *et al.* (3) report that the highly conserved *let-7*–LIN-41 heterochronic signaling pathway is responsible for part of the age-related decline in axon regeneration in the worm *Caenorhabditis elegans*.

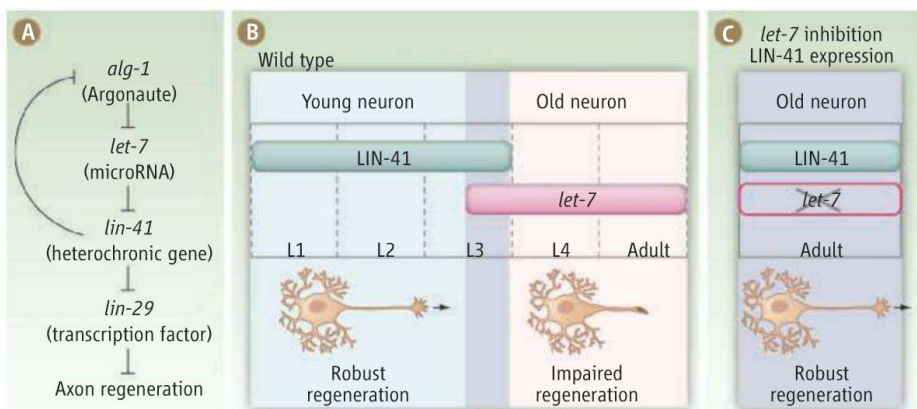
Zou *et al.* examined axon regeneration in mutants of *dcr-1* (dicer) and *alg-1* (argonaute), which are critical for microRNA biogenesis. The authors found that *alg-1* mutants showed improved axon regeneration. Out of 90 microRNAs tested, 10 that had late-onset expression correlated with the decline in axon regeneration. Only one, *let-7*, was affected by *alg-1* and expressed in the mechanosensory anterior ventral microtubule (AVM) neuron. *let-7* is a 21-nucleotide RNA that regulates the heterochronic gene *lin-41* (4), which in turn inhibits the LIN-29 transcription factor (5). Heterochronic genes cause shifts in the timing of developmental events primarily through changes in the timing of mitotic-associated differentiation events (6). The *let-7*–LIN-41 heterochronic pathway regulates larval-to-adult fate transitions (5). Zou *et al.* discovered

that this pathway also controls the axon regeneration ability of postmitotic neurons (see the figure). In larval animals, LIN-41 inhibits LIN-29 to keep axon regeneration robust in young neurons, while at the same time repressing ALG-1 function to inhibit *let-7* microRNA biogenesis. In adult animals, the signaling pathway somehow switches, possibly via a positive feedback loop between *let-7* and ALG-1 (7). *let-7* targets LIN-41, thus relieving inhibition of the LIN-29 transcription factor, which then targets unknown genes to repress axon regeneration in adult neurons.

A signaling pathway is implicated in the age-dependent decline of neuron regeneration.

via LIN-14, affects axon pathfinding of the AVM neuron partly by regulating the UNC-40 netrin receptor (10). ALG-1 also regulates the biogenesis of *lin-4* (in addition to *let-7*) and shows a similar AVM axon pathfinding phenotype. Yet, the finding by Zou *et al.* that *lin-4* shows no axon regeneration phenotype is perplexing given the pathfinding requirements for regenerating axons and the interconnected heterochronic signaling pathways.

Are there other genes that affect the age-related decline in axon regeneration? The kinase DLK-1 has a potent effect on axon



Regulating neuron regeneration. (A) Genetic pathway controlling regeneration competency. (B) LIN-41 is expressed in early larval stages, when young neurons regenerate efficiently. Onset of *let-7* expression correlates with later larval and adult stages. Increasing *let-7* expression in aging neurons inhibits regeneration via inhibition of LIN-41 and disinhibition of LIN-29. (C) Regeneration in older neurons can be restored by disrupting *let-7* expression or increasing LIN-41 expression through adulthood.

The most surprising feature of this pathway is that transiently disrupting *let-7* function at the time of axotomy in older neurons still leads to an improvement in axon regeneration. Thus, an older postmitotic neuron can be rapidly converted to a younger, more robust state. Although encouraging and relevant for the potential therapeutic application of this pathway to spinal cord injury, this is not the first finding of heterochronic genes acting cell autonomously in postmitotic neurons. LIN-14 regulates the timing of synaptic remodeling during the maturation of a specific class of motor neurons in *C. elegans* (8). This result is particularly interesting because LIN-14 is regulated by the *lin-4* microRNA, which in turn can regulate *let-7* (9). *lin-4*,

regeneration, and its overexpression rescues the age-related decline in axon regeneration ability in DD motor neurons (11). However, it is not known if there is a corresponding decline in DLK-1 activity with age. Zou *et al.* found that *lin-41* could not suppress the DLK-1 pathway. It will be interesting to determine if these two pathways act in parallel to further delay the decline of axon regeneration. A recent screen found several genes that showed improved axon regeneration and are obvious candidates to test for their influence on its age-related decline (12).

What is the potential for this heterochronic pathway to function in humans? The LIN-41–TRIM71 regulatory circuit is present in mammals and is similarly regulated by *let-7* (5,

Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112-0840, USA. E-mail: paolanix1@gmail.com; bastiani@biology.utah.edu

13, 14). *let-7*, LIN-41, and LIN-29 are being extensively studied in mice for their role in controlling cell proliferation and differentiation (15). Just as in *C. elegans*, mammalian LIN-41 expression decreases with age and is associated with decreased proliferation and increased differentiation of neural tissue (13, 16). Thus, it is likely that this pathway is functioning in a similar way to inhibit axon regeneration in postmitotic mammalian neurons.

Why would older neurons decrease their ability to regenerate axons? In *C. elegans*, the steep decline in axon regeneration ability is associated with a cessation of body growth and the beginning of reproduction. Perhaps there is a diversion of energy toward reproduction or an advantage to stabilizing basic

neural circuits. In the mammalian central nervous system, the initial loss of axon regeneration ability occurs very early in development and suggests that axon stabilization may be the more likely rationale. The reason will remain a mystery until more of the genes regulating the age-dependent decline in axon regeneration are identified and their functions in aging neurons understood. Zou *et al.* have taken an important first step toward understanding aging neurons with the identification of the heterochronic *let-7*-LIN-41 pathway in *C. elegans*.

References

1. D. F. Chen, S. Jhaveri, G. E. Schneider, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 7287 (1995).
2. M. Blackmore, P. C. Letourneau, *J. Neurobiol.* **66**, 348 (2006).

3. Y. Zou *et al.*, *Science* **340**, 372 (2013).
4. B. J. Reinhart *et al.*, *Nature* **403**, 901 (2000).
5. F. J. Slack *et al.*, *Mol. Cell* **5**, 659 (2000).
6. V. Ambros, H. R. Horvitz, *Science* **226**, 409 (1984).
7. D. G. Zisoulis, Z. S. Kai, R. K. Chang, A. E. Pasquinelli, *Nature* **486**, 541 (2012).
8. S. J. Hallam, Y. Jin, *Nature* **395**, 78 (1998).
9. D. T. Harris, H. R. Horvitz, *Development* **138**, 4051 (2011).
10. Y. Zou, H. Chiu, D. Domenger, C. F. Chuang, C. Chang, *Sci. Signal.* **5**, ra43 (2012).
11. M. Hammarlund, P. Nix, L. Hauth, E. M. Jorgensen, M. Bastiani, *Science* **323**, 802 (2009).
12. L. Chen *et al.*, *Neuron* **71**, 1043 (2011).
13. B. R. M. Schulman, A. Esquela-Kerscher, F. J. Slack, *Dev. Dyn.* **234**, 1046 (2005).
14. Y.-C. Lin *et al.*, *Mol. Biol. Evol.* **24**, 2525 (2007).
15. M. Ecsedi, H. Grosshans, *Genes Dev.* **27**, 581 (2013).
16. J. Chen, F. Lai, L. Niswander, *Genes Dev.* **26**, 803 (2012).

10.1126/science.1237921

APPLIED PHYSICS

Polarization Traffic Control for Surface Plasmons

Andrey E. Miroshnichenko and Yuri S. Kivshar

A stone thrown into water produces waves that evenly spread out in all directions along the surface, but the symmetry patterns of the propagating waves can be changed if, for example, two stones are thrown in at different places and times. Similarly, surface plasmon polaritons—electromagnetic waves bounded to a metal-dielectric interface—that are excited by different sources can also interfere with each other (1). These “surface plasmons,” as they are commonly called, inherit one important property from photons: They are

polarized, which turns out to be important for controlling their interference. On page 331 of this issue, Lin *et al.* (2) demonstrate a somewhat unexpected property of surface plasmons. Carefully designed nanostructures can excite plasmons propagating in the direction uniquely defined by the type of polarization of the incident light. On page 328, Rodríguez-Fortuño *et al.* (3) show that near-field effects in the interference phenomena also allow unidirectional propagation of surface plasmons in a symmetric structure excited by circularly polarized light.

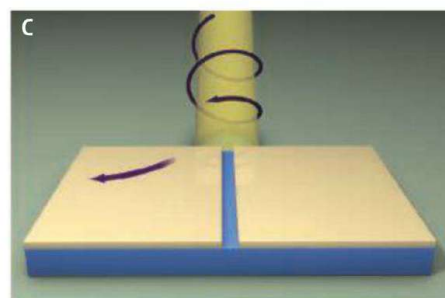
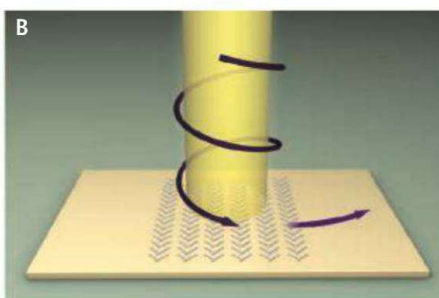
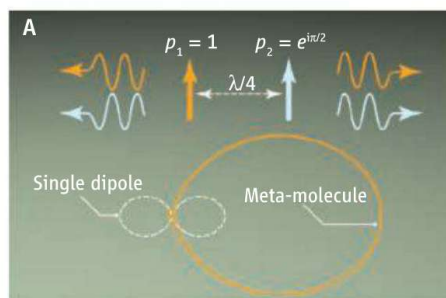
Surface plasmons are sustained by the collective electronic oscillations (plasma waves) in the metal in proximity to the interface. Plasmons are essentially two-

Metal surfaces structured at a scale below the wavelength of light create devices that control plasmon beam direction through their polarization.

dimensional waves whose field components decay exponentially with distance from the surface. This sensitivity to distance is exploited in molecule diagnostics and biosensing applications when molecules bind to a surface that supports surface plasmons (e.g., gold).

Surface plasmon beams can also carry energy from one location on the surface to another, and the control of surface plasmon propagation is crucially important for next-generation plasmonic circuitry. Future technologies will demand an increase in photonic integration that can be achieved only by subwavelength structuring with embedded functionalities at the level of material rather than an entire chip—that is, to cre-

Nonlinear Physics Centre, Australian National University, Canberra ACT 0200, Australia. E-mail: aem124@physics.anu.edu.au; ysk124@physics.anu.edu.au



Pointing surface plasmons. (A) The emission pattern generated by two electric dipoles separated by a quarter of a wavelength with a relative $\pi/2$ phase shift is shown and is compared to that for a single dipole. (B) Schematic view of the unidirectional propagation of surface plasmons excited by arrays of aperture pairs fabricated in a gold film. Circularly polarized light interacts with a structured surface in

such a way that the direction of the excited plasmon beam depends on the incident beam handedness. A change of the light polarization from right to left handedness will send the plasmons in the opposite directions. (C) Schematic view of the incident elliptically polarized light onto a gold metallic slit at nearly grazing angle, which excites a unidirectional plasmon beam depending on its handedness.

ate “metadevices” (4). Photonic crystals and metamaterials are examples of such structured materials that have properties not available in nature. Other examples include metasurfaces created by a lattice of V-shaped nanoantennas (5) and engineered nanoscale phase gratings that enable the generation of the Airy plasmons that can substantially bend the beam over its propagation length (6, 7), as well as other types of nondiffracting surface plasmon waves (8).

Lin *et al.* fabricated a metasurface in a gold film as arrays of “meta-molecules” composed of two perpendicular rectangular apertures. Each of these apertures acts as an electric dipole source and generates surface plasmons under an external light illumination. An elongated rectangular shape of each aperture caused the excitation of surface plasmons to become polarization dependent. When two perpendicular apertures are placed in close proximity, the surface-plasmon interference becomes independent of the direction of linear polarized light but dependent on the handedness of circular polarized light.

The basic operation principle of this type of device is shown in the figure, panel A. Two electric dipoles separated by a quarter of a wavelength with an additional $\pi/2$ phase shift between them generate unidirectional radiation pattern as a result of the in-phase wave interference in one direction and out-of-phase interference in the opposite direction. Similarly, the unidirectional radiation pattern can be obtained from the superposition of electric and magnetic dipoles (9). The interference of two emitters is realized by Lin *et al.* for a meta-molecule consisting of two orthogonal rectangular apertures separated by a quarter of a surface plasmon wavelength. The arrangement of multiple parallel rows of apertures formed by the meta-molecules acts like a grating and efficiently couples the incident light to surface plasmons.

However, because of the specific arrangement of the apertures in pairs, the coupling remains unidirectional, such that the direction of the excited plasmon beam depends on the incident beam handedness (see the figure, panel B). This property enables directional coupling of orthogonal polarizations of the incident light into counterpropagating surface plasmons. This idea was further applied to circular metallic structures that generate radially convergent or divergent surface plasmons depending on the polarization state of the incident light. Such excitation is necessary for focusing of surface plasmons and for “on demand” forma-

tion of hot spots (10–12). It also allows us to study effectively in-plane scattering phenomena with localized objects (13).

Rodríguez-Fortuño *et al.* discuss the crucial importance of the near-field effects in the interference of vectorial fields. They demonstrate experimentally that an elliptically polarized dipole can produce either destructive or constructive interference of different evanescent components in its near field, eventually controlling directionality of the propagating modes in a plasmonic waveguide (see the figure, panel C). This near-field interference provides a fundamentally different approach to unidirectional excitation and propagation of guided waves with broadband nonresonant characteristics and another opportunity to achieve the polarization control of the directionality of the plasmon beams.

We believe these studies will stimulate more effort toward a better understanding and use of polarization-selective and near-field effects in the physics of surface plasmons. They will open new opportunities for

unidirectional manipulation of surface plasmons, including interconnects and single plasmon detection, and for their application in areas such as integrated quantum information processing.

References and Notes

1. B. Hecht, H. Bielefeldt, L. Novotny, Y. Inouye, D. W. Pohl, *Phys. Rev. Lett.* **77**, 1889 (1996).
2. J. Lin *et al.*, *Science* **340**, 331 (2013).
3. F. J. Rodríguez-Fortuño *et al.*, *Science* **340**, 328 (2013).
4. N. I. Zheludev, Y. S. Kivshar, *Nat. Mater.* **11**, 917 (2012).
5. N. Yu *et al.*, *Science* **334**, 333 (2011).
6. A. Minovich *et al.*, *Phys. Rev. Lett.* **107**, 116802 (2011).
7. L. Li, T. Li, S. M. Wang, C. Zhang, S. N. Zhu, *Phys. Rev. Lett.* **107**, 126804 (2011).
8. J. Lin *et al.*, *Phys. Rev. Lett.* **109**, 093904 (2012).
9. W. Liu, A. E. Miroshnichenko, D. N. Neshev, Y. S. Kivshar, *ACS Nano* **6**, 5489 (2012).
10. L. Yin *et al.*, *Nano Lett.* **5**, 1399 (2005).
11. F. López-Tejeda *et al.*, *Nat. Phys.* **3**, 324 (2007).
12. L. Li, T. Li, S. Wang, S. Zhu, X. Zhang, *Nano Lett.* **11**, 4357 (2011).
13. N. Rotenberg *et al.*, *Phys. Rev. Lett.* **108**, 127402 (2012).

Acknowledgments: We thank D. Neshev for useful discussions and important references.

10.1126/science.1236154

EVOLUTION

Great Apes and Zoonoses

Paul M. Sharp¹, Julian C. Rayner², Beatrice H. Hahn³

Comparing the origins of AIDS and malaria may provide insight for gauging the prospect of future pathogen transmissions from apes to humans.

Two of the most widespread and virulent diseases of modern humans appear to have originated in our closest relatives, the African apes. There is evidence that the parasite *Plasmodium falciparum*, the cause of malaria in humans, is of gorilla origin (1, 2), whereas the pandemic form of human immunodeficiency virus type 1 (HIV-1), the cause of acquired immune deficiency syndrome (AIDS), stems from a lentivirus infection of chimpanzees (3). Is their great ape origin a coincidence, or are there important parallels that could provide insight into the prospects of future zoonoses in which other pathogens from the great apes might successfully colonize humans?

¹Institute of Evolutionary Biology, University of Edinburgh, Edinburgh EH9 3JT, UK. ²Malaria Programme, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK. ³Departments of Medicine and Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA. E-mail: bhahn@upenn.edu

Pathogens must interact with a large number of host proteins to replicate in infected cells and thus tend to spread more readily between closely related hosts (4). Hence, the precursors of HIV-1 and *P. falciparum* that originated in great apes were likely better suited to infect humans than lentiviruses or malaria parasites from other mammals. However, despite substantial exposure and possibly numerous cross-species transfers, humans appear to have been successfully colonized by each of these pathogens only once. This implies that even ape pathogens have to overcome substantial adaptive hurdles to become established in the human host. For example, it has long been assumed that a human-specific mutation in the sialic acid biosynthetic pathway protects humans from infection by the species of *Plasmodium* that infect apes (5). However, not all *P. falciparum* parasites use sialic acids to invade erythrocytes, whereas an interaction between a parasite protein and the Ok blood group antigen basigin appears to be

an obligatory step in this process (6). The ape homologs of basigin are quite divergent, and so it is likely that the gorilla precursor of *P. falciparum* had to adapt to spread efficiently in the human host. Similarly, lentiviruses, such as HIV-1 and its chimpanzee precursor—a strain of simian immunodeficiency virus called SIVcpz—are susceptible to various host-specific restriction factors, including tetherin, a protein that inhibits the egress of virions from infected cells. There are four groups of HIV-1 (called M, N, O, and P), which each arose from an independent ape-to-human transmission event; however, only group M viruses have achieved full anti-tetherin adaptation, and only this group has become pandemic (7).

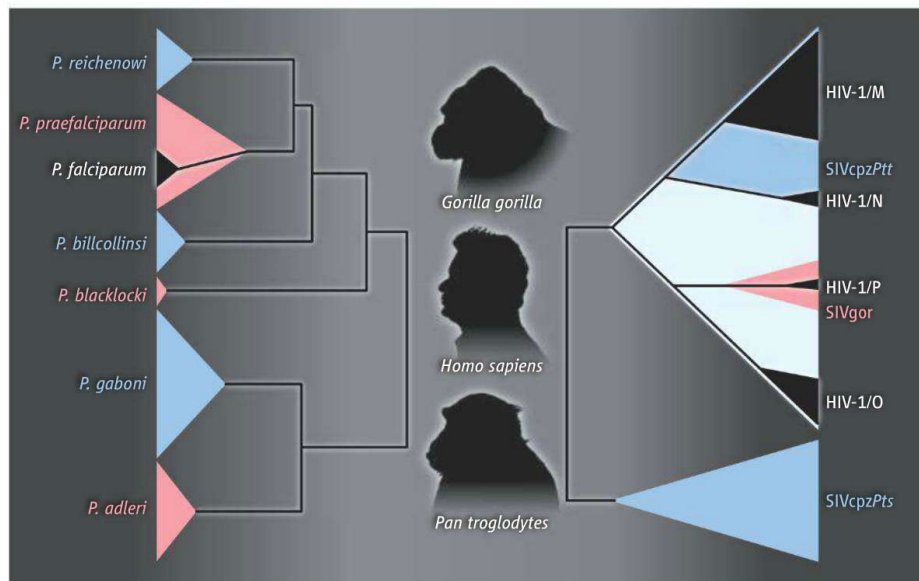
That only a small subset of ape pathogens has successfully colonized humans highlights another point. Although two subspecies of chimpanzees, *Pan troglodytes troglodytes* and *P. t. schweinfurthii*, are each endemically infected by distinct forms of SIVcpz, only strains from the former have been identified in humans (8) (see the figure). Similarly, the ancestor of *P. falciparum* represents just one of at least six distinct *Plasmodium* species within the subgenus *Laverania* that infect either western gorillas or chimpanzees (1). Thus, at least in these two cases, African apes harbor a much greater variety of related pathogens than have been found in humans, raising the question: Are the seemingly

unsuccessful pathogens incapable of infecting humans, or do they have the same potential to cross-infect and adapt, but have not yet had the opportunity? The latter possibility has obvious public health implications.

In addition to *Laverania* parasites, wild apes also harbor close relatives of *P. vivax*, *P. malariae*, and *P. ovale* (1, 9), raising the possibility that each of these human parasites was also acquired from apes. The case of *P. vivax* is particularly intriguing: The closest known relatives of *P. vivax* had been thought to be parasites infecting macaques (which are monkeys, not apes) in Southeast Asia, suggesting that *P. vivax* may have originated in this region (10). However, a mutation that confers protection against *P. vivax* by inhibiting expression of its receptor, the Duffy antigen present on the surface of red blood cells, is not found in Asian populations. This mutation has a frequency nearing 100% in human populations in west and central Africa, which has been taken to indicate that *P. vivax* is more likely to have originated there (11). That wild African apes harbor closely related *Plasmodium* strains is consistent with this interpretation (12). Thus, searching for pathogens related to known infectious agents could reveal additional potential zoonoses. A case in point is HIV-2, a lentivirus that originated in sooty mangabeys (monkeys found in west Africa), which was discovered to also cause human AIDS while screening for HIV-1 (8).

The ability of ape pathogens to colonize humans also appears to have depended on a number of historical (and even prehistorical) events. Timing the origin of *P. falciparum* in humans is problematic because of difficulties calibrating the rate of molecular evolutionary change in *Plasmodium*. Although it has been suggested that *P. falciparum* emerged out of Africa with modern humans, around 60,000 years ago (13), others have argued that *P. falciparum* could only have been sustained in the human population within the last 10,000 years, after the advent of agriculture and the concomitant changes in human (and mosquito) population density (14). By contrast, pandemic HIV-1 emerged much more recently, around a century ago (8). Again, this timing would reflect not the opportunity for cross-species transmission, but rather the potential for onward spread, provided in this case by social changes associated with colonial rule and the rapid growth of west central African cities (15). Thus, the emergence of both *P. falciparum* and HIV-1 were likely contingent upon changes in human demography and behavior that opened new niches for these parasites.

Although the circumstances that aided the initial expansions of *P. falciparum* and HIV-1 may no longer be relevant, other human-controlled factors may open new niches for ape-derived pathogens in the future. For example, efforts to eliminate *P. falciparum* malaria in Africa will undoubtedly reduce the density of human *Plasmodium* infections, but this may ease the competitive burden for any newly transferred parasites. The monitoring of human populations that are at a higher possible risk, such as hunters and forest dwellers, may detect new cross-species transmissions. Such studies require new diagnostic tools to detect emerging pathogens. An example is the macaque parasite *P. knowlesi*, which went unrecognized as a public health threat (although it was long known to infect humans) until molecular screening revealed hundreds of zoonotic transmissions each year (16). It will also be important to study the ape precursors of the human pathogens in their natural hosts. For SIVcpz such studies have already produced invaluable information about its susceptibility to host restriction factors, pathogenic potential, and adaptability (7, 8); as yet nothing is known about the course of *Plasmodium* infections in apes. Such studies may also address the intriguing question of why successful pathogen transfer from apes to humans is a rare event. And although it is difficult to predict whether or not newly discovered ape pathogens have the potential



Ape reservoir. (Left) The subgenus *Laverania* contains six *Plasmodium* species, three that infect chimpanzees (blue) and three that infect gorillas (pink); only one of these has given rise to a human parasite (*P. falciparum*; black) (12). (Right) There are two major SIVcpz lineages that infect the central (upper blue clade) and eastern (lower blue clade) subspecies of chimpanzees, respectively. SIVcpzPtt strains from central chimpanzees have crossed the species barrier to humans, generating HIV-1 groups M and N (black), and to gorillas, generating SIVgor (pink). Group P is of likely gorilla origin, whereas the ape precursor of group O has not yet been defined (light blue) (8). Despite widespread distribution among eastern chimpanzees, SIVcpzPts has not been found in humans.

CREDIT: C. BICKEL/SCIENCE

to infect humans, new diagnostic tools will place researchers in a better position to provide advance warning that could benefit any subsequent public health response.

References

1. W. Liu *et al.*, *Nature* **467**, 420 (2010).
2. A. Ayoub *et al.*, *Int. J. Parasitol.* **42**, 709 (2012).
3. B. F. Keele *et al.*, *Science* **313**, 523 (2006).
4. T. J. Davies, A. B. Pedersen, *Proc. Biol. Sci.* **275**, 1695 (2008).
5. M. J. Martin, J. C. Rayner, P. Gagneux, J. W. Barnwell, A. Varki, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 12819 (2005).
6. C. Crosnier *et al.*, *Nature* **480**, 534 (2011).
7. D. T. Evans *et al.*, *Trends Microbiol.* **18**, 388 (2010).
8. P. M. Sharp, B. H. Hahn, *Cold Spring Harb. Perspect. Med.* **1**, a006841 (2011).
9. L. Duval *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 10561 (2010).
10. A. A. Escalante *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 1980 (2005).
11. R. Carter, *Trends Parasitol.* **19**, 214 (2003).
12. J. C. Rayner, W. Liu, M. Peeters, P. M. Sharp, B. H. Hahn, *Trends Parasitol.* **27**, 222 (2011).
13. K. Tanabe *et al.*, *Curr. Biol.* **20**, 1283 (2010).
14. R. Carter, K. N. Mendis, *Clin. Microbiol. Rev.* **15**, 564 (2002).
15. J. Pepin, *The Origins of AIDS* (Cambridge Univ. Press, Cambridge, 2011).
16. A. Kantele, T. S. Jokiranta, *Clin. Infect. Dis.* **52**, 1356 (2011).

10.1126/science.1236958

MATERIALS SCIENCE

Pursuing Near-Zero Response

Nader Engheta

In most wave phenomena, the interplay between the spatial and temporal features of a wave is influenced by the medium in which the wave propagates. For example, the wavelength λ (a spatial feature) and the frequency f (a temporal feature) of a propagating signal are related via the phase velocity v of the wave in the medium as $v = f\lambda$. For electromagnetic waves such as radio, microwave, and optical waves, the phase velocity is determined by the medium's electromagnetic parameters of permittivity ϵ and permeability μ , which is then given as $v = 1 / \sqrt{\epsilon\mu}$. When a wave interacts with a structure embedded in a host medium, both these temporal and spatial features play key roles in determining the scattering response of the structure. The recent development of a class of metamaterials in which the electric (ϵ) and magnetic (μ) properties can be tuned by design is providing a platform to engineer optical devices with unconventional properties.

The frequency of the wave tells us how the structure's materials behave, whereas its wavelength in the host medium is the yardstick by which the physical dimensions of the structure affect the wave-structure interaction. These two aspects of interaction are often closely intertwined because any physical structure is made of a material and has certain physical dimensions. Of course, as we go to higher frequencies, wavelengths are generally shortened. However, metamaterials allow the link between the frequency and the wavelength to be relaxed. If either the permittivity or the permeability (or both) can be designed to be relatively low values near zero, the phase velocity of the wave will approach a very high value, resulting in long wavelengths at high frequencies (see the figure, panel A).

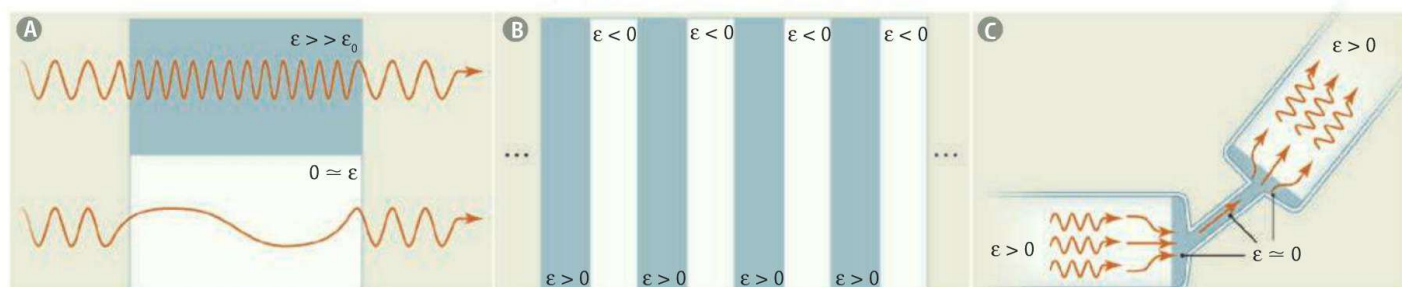
The class of materials in which the relative permittivity ϵ attains near-zero values around a given frequency is called Epsilon-Near-Zero (ENZ) metamaterials (1). When the operating frequency is near the plasma frequency of transparent conducting oxides [e.g., indium-tin-oxide (ITO)] (2), the real part of the permittivity gets near zero

A class of metamaterials designed with low permittivity provides a platform for developing optical devices with unconventional properties.

(whereas its imaginary part is small). Alternatively, metamaterials can be designed to achieve effective permittivity near zero by mixing constituent materials with permittivity-positive (oxides) and permittivity-negative (metal) structures, such as the stacks of thin layers of pairs of such structures (see the figure, panel B). Another way to imitate propagation in an ENZ medium is to exploit the structural dispersion of metal-clad waveguides near their cut-off frequencies (3, 4).

ENZ metamaterials offer unprecedented wave properties. One such property is the phenomenon of supercoupling (1), in which the wave in one waveguide can efficiently tunnel through a very narrow, ENZ-filled channel to reach another waveguide, regardless of the channel's length, shape, bending and twisting, and the relative orientation of the two waveguides (see the figure, panel C). Counterintuitively, the narrower the channel's height, the better the tunneling. The consequence of this unusual tunneling is threefold: (i) because the energy has to squeeze through the narrow channel with no reflection, the wave intensity is enhanced along the entire narrow channel, and such

University of Pennsylvania, Department of Electrical and Systems Engineering, Philadelphia, PA 19104, USA.
E-mail: engheta@ee.upenn.edu



Features of ENZ metamaterials. (A) The wavelength can be “stretched” within materials with low permittivity, whereas for high permittivity it is compressed. (B) A judicious mixture of a permittivity-positive and a permittivity-negative constituent structure may provide an effectively ENZ metamaterial.

(C) Supercoupling phenomenon in ENZ-filled ultranarrow channels between two similar, but arbitrarily oriented, waveguides, in which an efficient tunneling occurs regardless of the length, shape, bending, and twisting of the ultranarrow channel.

enhancement is inversely proportional to the ratio of the channel's height to the port waveguide's height; (ii) because the wavelength in the ENZ region is very long due to the low value of permittivity, the enhanced intensity maintains an approximately uniform phase along the entire channel's length; and (iii) this enhancement and its uniformity along the channel are essentially independent of the length, shape, and bending of the channel. Because of its high wave intensity enhancement over an extended region, this phenomenon has been studied for boosting nonlinear effects (5) and second-harmonic generation and has also been used for enhancing the photon density of state for emitters embedded in such ENZ structures (6, 7). This platform has also been proposed for sensing a defect in the channel (8), thus opening the possibility for fluidic- and biosensing applications. The supercoupling concept has now been extended to other wave phenomena, such as acoustic and matter waves (9).

Because the permittivity of these materials is near zero, the electric displacement vector may also be near zero for a finite electric field. Therefore, an ENZ material can be used as a platform for "shielding" the displacement current, just as a dielectric insulator may shield the conduction current in a metallic wire (10, 11).

ENZ structures may provide a useful mechanism for enhancing the effects of

nonreciprocity and time-reversal symmetry breaking, when they are combined with magneto-optical materials. Such composites may favorably change the balance between the parameter of the magneto-optical activity and the dielectric parameters, causing pronounced nonreciprocal effects such as wave isolation based on circular polarization and Faraday rotation (12).

In conventional media, random displacement of tiny scatterers causes temporal decoherence of the scattered signals. Because the wavelength is increased in ENZ media, random fluctuation of scatterers embedded in these media may lead to decreased incoherence and thus may maintain a more coherent signal compared with conventional materials.

Finally, when the permeability μ of ENZ metamaterials is also near zero, the refractive index of the medium again approaches near-zero value; however, here the wave impedance is preserved (13–15). In such a scenario, a large volume of this medium may occupy appreciable physical space, while still being tiny from the wave point of view (because the wavelength has become long). This will provide an interesting possibility to "stretch" and "open up" a part of a physical system by expanding the region and filling it with index-near-zero medium. Such "opening up" of the space should not affect the wave interaction in the external region but could provide a platform to insert objects and elements

that ordinarily would not fit in the region with standard materials. This concept may open doors to novel spectroscopy of nanostructures in which the effects of the size of the object may be decoupled from its material dispersion.

References and Notes

1. M. G. Silveirinha, N. Engheta, *Phys. Rev. Lett.* **97**, 157403 (2006).
2. A. Boltasseva, H. A. Atwater, *Science* **331**, 290 (2011).
3. B. Edwards, A. Alù, M. E. Young, M. Silveirinha, N. Engheta, *Phys. Rev. Lett.* **100**, 033903 (2008).
4. I. I. Smolyaninov, V. N. Smolyaninova, A. V. Kildishev, V. M. Shalaev, *Phys. Rev. Lett.* **102**, 213901 (2009).
5. C. Argyropoulos, P.-Y. Chen, G. D'Aguanno, N. Engheta, A. Alu, *Phys. Rev. B* **85**, 045129 (2012).
6. A. Alù, N. Engheta, *Phys. Rev. Lett.* **103**, 043902 (2009).
7. E. J. R. Vespeur, T. Coenen, H. Caglayan, N. Engheta, A. Polman, *Phys. Rev. Lett.* **110**, 013902 (2013).
8. A. Alu, N. Engheta, *Phys. Rev. B* **78**, 045102 (2008).
9. R. Fleury, A. Alu, *Appl. Phys. A* **109**, 781 (2012).
10. A. Alù, N. Engheta, *Phys. Rev. Lett.* **103**, 143902 (2009).
11. B. Edwards, N. Engheta, *Phys. Rev. Lett.* **108**, 193902 (2012).
12. A. R. Davoyan, A. M. Mahmoud, N. Engheta, *Opt. Express* **21**, 3279 (2013).
13. R. W. Ziolkowski, *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **70**, 046608 (2004).
14. N. M. Litchinitser, A. I. Maimistov, I. R. Gabitov, R. Z. Sagdeev, V. M. Shalaev, *Opt. Lett.* **33**, 2350 (2008).
15. X. Huang, Y. Lai, Z. H. Hang, H. Zheng, C. T. Chan, *Nat. Mater.* **10**, 582 (2011).

Acknowledgments: This work is supported in part by the U.S. Office of Naval Research Multidisciplinary University Research Initiatives grant N00014-10-1-0942.

10.1126/science.1235589

ECOLOGY

Fire in the Ocean

C. A. Masiello¹ and P. Louchouart^{2,3}

One of the great challenges of modern organic geochemistry is the determination of the chemical composition of Earth's largest active carbon pools: marine sediments, soils, and dissolved organic carbon (DOC) in both marine and terrestrial systems. Together these reservoirs are much larger than the atmospheric CO₂ pool, and they exchange carbon with the atmosphere, making them potential CO₂ sources or sinks in a changing climate. Rivers play a central role in the carbon exchange between all these reservoirs.

On page 345 of this issue, Jaffé *et al.* (1) shed new light on the chemical composition of riverine DOC.

Earth's organic carbon reservoirs are thought to derive from living terrestrial and marine organic matter, altered through biotic and abiotic processes. However, once organic matter enters soils, rivers, and the ocean, its chemistry diverges rapidly from the easily characterized chemistry of fresh plant matter (2). Fire may be partly responsible for this: Burning alters the chemistry of biomass, rendering its organic chemical composition undetectable by standard analytical techniques. Black carbon (BC), carbonaceous material altered by fire, can be transported globally through the atmosphere, rivers, and oceans. Because exposure to fire can reduce biomass decompos-

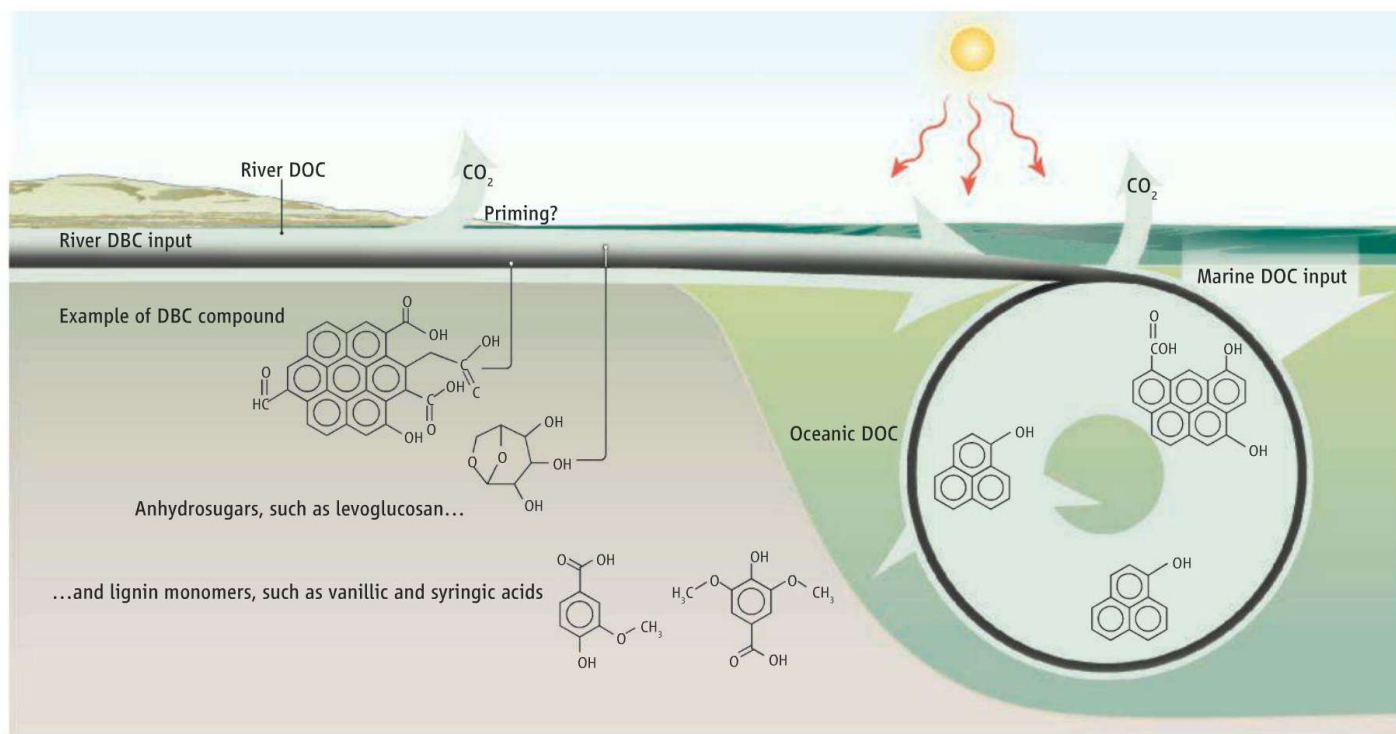
Exposure to fire alters the properties of dissolved organic carbon in ways that affect how it decomposes in rivers and in the ocean.

ability, BC becomes an increasing fraction of carbon pools as they age.

Quantitating BC is analytically hard, and it has been particularly challenging to measure dissolved BC (DBC). Jaffé *et al.* have applied a novel DBC method to a large suite of river samples. With this method, which measures a highly recalcitrant fraction of DBC that is enriched in condensed polycyclic aromatic units, the authors identified more than 10% of DOC in rivers as DBC. It does not detect other DBC fractions such as levoglucosan (3). This means that the 10% value reported by Jaffé *et al.* is a low estimate of fire-altered DOC in rivers.

An even more interesting result is the strong correlation between DBC and DOC concentrations in their river samples, indicating that fire does not create a distinct

¹Department of Earth Science, Rice University, 6100 Main Street, MS 126, Houston, TX 77005, USA. ²Department of Marine Science, Texas A&M University at Galveston, Galveston, TX 77553, USA. ³Department of Oceanography, Texas A&M University, College Station, TX 77853, USA. E-mail: masiello@rice.edu



From rivers to ocean. Dissolved organic carbon (DOC) from rivers enters the ocean where it mixes into the larger marine DOC pool that travels through the ocean's thermohaline circulation. Jaffé *et al.* (1) show that at least 10% of the DOC entering the ocean is dissolved black carbon (DBC). As river DBC enters the ocean, the photoreactive and bioreactive components are degraded (10, 14), symbolized by

the narrowing of the DBC band. Some reactive DBC components may also prime the decomposition of bulk DOC. A thin vector of DBC runs through the conveyor loop, representing the ~4 cycles of circulation needed to decompose DBC (8). More condensed aromatic DBC is photo-oxidized in the surface ocean, leading to the accumulation of smaller DBC fragments in the deep ocean (14, 15).

fraction of DOC with unusual properties. Instead, in the 27 rivers and wetlands analyzed by Jaffé *et al.*, DBC behaves like the bulk DOC pool, which suggests that fire-altered carbon is an intrinsic component of the reservoir.

This study is part of a growing body of work deepening our understanding of the decomposability of BC. A simple model (2, 4) proposed a BC combustion continuum, where higher-temperature combustion creates larger aromatic ring clusters with longer environmental residence times. In terrestrial systems larger ring structures do appear to be more stable (5, 6), but once DBC leaves soils, larger ring clusters seem to be more photoreactive (7), leading to the accumulation of smaller aromatic structures in the oceans (see the figure) (8).

The chemical heterogeneity of DBC leads to a reactivity that does not mesh simply with the black carbon continuum model. Biomass combustion leads to structurally variable residues (9) that may degrade faster or slower than their precursor biomass (10). The soluble organics released by fire can be quite reactive (10, 11) and may further accelerate (prime) the decomposition of bulk DOC. Potential priming effects

induced by terrigenous DOC in inland and coastal waters have been identified as a gap in global carbon cycle models (12), and low molecular weight organics released from charcoals or from the photo-oxidation of larger DBC clusters may be priming substrates, impacting DOC cycling.

Research on the heterogeneity in BC environmental residence time is crucial for the widely applied carbon sequestration technique of biochar soil amendment. In this approach, biomass is converted into charcoal and added to soil, transferring carbon from fast-cycling biomass pools into the slow-cycling soil BC pool. Increased crop yields and altered soil hydrology have been observed in some biochar-amended systems (13). However, our understanding of the effectiveness of this technique is only as good as our understanding of the residence time of BC in soils, rivers, and the ocean.

Jaffé *et al.* mention that biochar soil amendment may have unintended consequences through increased transport of DBC into aquatic and marine systems, with downstream impacts on aquatic food webs. These possibilities must be taken seriously. The successful scaling-up of biochar soil amendment will require assessment of the

fate of biochar carbon both in the solid and dissolved phases. Jaffé *et al.*'s observation that the aromatic, dissolved fraction of BC behaves at least in part like bulk DOC may make tracking DBC that much easier.

References

1. R. Jaffé *et al.*, *Science* **340**, 345 (2013).
2. J. I. Hedges *et al.*, *Org. Geochem.* **31**, 945 (2000).
3. G. B. Hunsinger, S. Mitra, J. A. Warrick, C. R. Alexander, *J. Geophys. Res.* **113**, 14 (2008).
4. C. A. Masiello, *Mar. Chem.* **92**, 201 (2004).
5. A. R. Zimmerman, *Environ. Sci. Technol.* **44**, 1295 (2010).
6. O. R. Harvey *et al.*, *Environ. Sci. Technol.* **46**, 1415 (2012).
7. A. Stubbins, J. Niggemann, T. Dittmar, *Biogeosciences* **9**, 1661 (2012).
8. L. A. Ziolkowski, E. R. M. Druffel, *Geophys. Res. Lett.* **37**, L16601 (2010).
9. A. V. McBeath, R. J. Smernik, E. S. Krull, *Org. Geochem.* **55**, 38 (2013).
10. M. J. Norwood, P. Louchouart, L.-J. Kuo, O. R. Harvey, *Org. Geochem.* **56**, 111 (2013).
11. O. R. Harvey, B. E. Herbert, L.-J. Kuo, P. Louchouart, *Environ. Sci. Technol.* **46**, 10641 (2012).
12. T. S. Bianchi, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 19473 (2011).
13. S. P. Sohi, *Science* **338**, 1034 (2012).
14. R. G. M. Spencer *et al.*, *J. Geophys. Res.* **114**, G03010 (2009).
15. W. C. Hockaday, A. M. Grannas, S. Kim, P. G. Hatcher, *Geochim. Cosmochim. Acta* **71**, 3432 (2007).

10.1126/science.1237688

WEBINAR

Proteomics Meets Cellular Signaling

Exploring Post-Translational Modifications by Mass Spectrometry

REGISTER NOW!
webinar.sciencemag.org

Wednesday, May 1, 2013

12 noon Eastern, 9 a.m. Pacific, 5 p.m. UK, 6 p.m. CEST

Post-translational modifications (PTMs)—including phosphorylation, acetylation, and ubiquitination—are specific modifications that can alter the activity of an individual protein target. The cumulative effect of these small modifications is the regulation of large signaling pathways and networks within cells.

Mass spectrometry is a powerful tool for the identification of individual PTMs during the investigation of signaling networks. A new approach using antibody-based peptide enrichment for a class of PTMs combined with tandem mass spectrometry has been successfully used for global identification of hundreds to thousands of PTMs within a sample. In this webinar, examples of how the technology was applied to each of these PTM classes will be described.

During this webinar, viewers will:

- Learn about post-translational modifications and their role in cellular signaling
- Discover how antibody-based proteomics approaches can be applied to identify, study, and characterize known and novel modifications
- Hear specific examples of how these approaches are being applied to the dissection of cellular signaling pathways, target identification, validation, and biomarker discovery by industry and academic experts
- Ask our expert panel questions in real time, during the broadcast

Speakers



Chunaram Choudhary, Ph.D.,
The Novo Nordisk Foundation
Center for Protein Research



Cloud Paweletz, Ph.D.,
Dana Farber Cancer Institute



Vipin Suri, Ph.D.,
Sirtris, a GSK Company

Webinar sponsored by



Brought to you by the
**Science/AAAS Custom
Publishing Office**



Grand Challenges in Science Education

Use technology to improve pedagogy, management, and accountability.

Improve access to, and the quality of pre- and postprimary education.

Develop appropriate policies for regulating and supporting the private sector in education.

Develop an understanding of how individual differences in brain development interact with formal education.

Adapt learning pathways to individual needs.

Create online environments that use stored data from individual students to guide them to virtual experiments that are appropriate for their stage of understanding.

Determine the ideal balance between virtual and physical investigations for courses in different subject areas.

Identify the skills and strategies that teachers need to implement a science curriculum featuring virtual and physical laboratories.

Identify the underlying mechanisms that make some teacher professional development (PD) programs more effective than others.

Identify the kind of PD that will best prepare teachers to meet the challenges of the Next Generation Science Standards.

Harness new technologies and social media to make high-quality science PD available to all teachers.

Help students explore the personal relevance of science and integrate scientific knowledge into complex practical solutions.

Develop students' understanding of the social and institutional basis of scientific credibility.

Enable students to build on their own enduring, science-related interests.

Shift incentives to encourage education research on the real problems of practice as they exist in school settings.

Create a set of school districts where long-standing, multidisciplinary teams work together to identify effective improvements.

Create a culture within school systems that allows for meaningful experimentation.

Design valid and reliable assessments reflecting the integration of practices, cross-cutting concepts, and core ideas in science.

Use assessment results to establish an empirical evidence base regarding progressions in science proficiency across K–12.

Build and test tools and information systems that help teachers effectively use assessments to promote learning in the classroom.

INTRODUCTION

Plenty of Challenges for All

WHY SHOULD ANYONE WHO IS NOT A SCIENTIST CARE ABOUT SCIENCE EDUCATION?

Professional scientists and educators may find the question insulting. Every culture has struggled to find the most effective ways to teach the uninitiated and translate that learning into productive skills. But if students and parents around the world don't see the need for a high-quality education in science, technology, engineering, and mathematics (the so-called STEM fields), or mistakenly think that they are already receiving satisfactory teaching in those areas, then calls from the scientific community to improve STEM education will fall on deaf ears.

In this special issue of *Science*, we have invited experts to tell us what they think are the most important challenges facing science education. Through a mixture of News, Reviews, Perspectives, Education Forums, and an Editorial, we explore the obstacles to progress, be they within the classroom, across the school system, or in the larger social arena. We also offer substantive suggestions on how to proceed. For example, distance education, online simulations as educational aids, and social networking tools are already part of science education. Many university faculty members are working to upgrade centuries-old approaches to instruction. And, with a new emphasis on the practice of science, promising assessment tools are being developed to improve learning.

The challenges covered in this special issue will be familiar to those who have devoted their lives and livelihoods to improving education. There is a huge and expanding literature on these topics and many others not covered in these pages. Yet convincing the public of the importance of STEM education will require more than explaining what the research shows or finding ways to scale up best practices to reach the billions of students who are entitled to a high-quality education. For scientists, advances in science and technology arrive at such a rapid clip that last year's knowledge barely scratches the surface of what is needed next year. At the same time, larger and more diverse student populations clamor for access to knowledge. Not only will the scientific workforce for the 21st century need skills and knowledge we haven't even heard of yet, but all global citizens, whether in their doctor's office or in a polling booth, need to be better informed. Turning the fire of the natural curiosity of students into effective, flexible, and well-grounded outcomes will take a concerted effort by many different actors. Among them, scientists must play a central role.

This is another grand challenge, and one that the scientific community ignores at its peril.

— PAMELA J. HINES, JEFFREY MERVIS, MELISSA MCCARTNEY, BRAD WIBLE

Grand Challenges in Science Education

CONTENTS

News

- 292 Transformation Is Possible
if a University Really Cares

Reviews

- 297 The Challenge of Education and Learning in the Developing World
M. Kremer et al.
- 300 Understanding Neurocognitive Developmental Disorders Can Improve Education for All
B. Butterworth and Y. Kovas
- 305 Physical and Virtual Laboratories in Science and Engineering Education
T. de Jong et al.
- 310 Professional Development for Science Teachers
S. M. Wilson
- 314 Outside the Pipeline: Reimagining Science Education for Nonscientists
N. W. Feinstein et al.
- 317 Generating Improvement Through Research and Development in Education Systems
M. S. Donovan
- 320 Proficiency in Science: Assessment Challenges and Opportunities
J. W. Pellegrino

Perspectives

- 309 Teacherpreneurs: A Bold Brand of Teacher Leadership for 21st-Century Teaching and Learning
B. Berry
- 313 A Business View on U.S. Education
R. Stephens and M. Richey

See also Editorial p. 249; Education Forums pp. 276 and 278; Science Signaling, Science Careers, Science Podcast, and more at www.sciencemag.org/special/education2013

Science



GRAND CHALLENGE: UNDERGRADUATE TEACHING

Transformation Is Possible If a University Really Cares

The same attention to scientific detail that led to his Nobel Prize is helping Carl Wieman improve how undergraduates learn science

The way that most research universities across North America teach science to undergraduates is worse than ineffective, says Carl Wieman. It's unscientific.

A Nobel Prize-winning physicist turned science educator, Wieman doesn't understand why institutions of higher education

would disregard decades of research showing the superiority of student-centered, active learning over the traditional 50-minute lecture. Using that outdated approach, he says, means universities are squandering talent at a time when U.S. higher education is being criticized for not turning out

enough science-savvy graduates to keep the country competitive.

Wieman has spent the past 15 years applying the science of learning to how undergraduate science courses are taught. First at the University of Colorado, Boulder, (colorado.edu/sei) and, more recently, at the University of British Columbia (UBC), Vancouver, in Canada (cwsei.ubc.ca), Wieman and his colleagues have made impressive strides in changing how individual faculty members teach. Those changes, within individual courses, have translated into big improvements in student learning.

Those courses are offered by academic departments, which are his real target. Departments define the reward structure

CREDIT: CASEY A. CASS/UNIVERSITY OF COLORADO



Engaged instruction. Carl Wieman uses active learning tools to teach an undergraduate course at the University of Colorado in 2001.

for faculty members through their authority to hire, promote, and grant tenure, he says. So the best way to sustain improvements in teaching and learning is to get departments to buy into the need to change the courses that they offer. And that's begun to happen at UBC, one of Canada's elite universities.

Wieman's passion for the subject, combined with his stature as a Nobelist, has focused national attention on the high attrition rate among students who declare an interest in earning a degree in a STEM (science, technology, engineering, and mathematics) field. It's one of the biggest impediments to any effort to train more scientists and engineers. "I think Carl, more than anybody else, put a spotlight on the need to improve under-

graduate education," says Subra Suresh, who last month stepped down as director of the National Science Foundation (NSF). "It wasn't a surprise to universities, but his work has highlighted the problem."

Colleagues also laud Wieman's rigorous approach to reform. "I have an incredible amount of respect for his deep commitment to the evidence," says Susan Singer, head of undergraduate education at NSF and a national leader in reforming undergraduate biology education. "Carl is someone who digs in and really wants to know."

Notwithstanding his success at Colorado and UBC, Wieman has made much less progress toward another of his goals:

time and money on research."

Wieman pushed the idea at numerous meetings with other government science officials and academic leaders. But they recoiled in horror at the prospect of what they viewed as another unfunded federal mandate. They prefer a 5-year effort begun last year by the 62-member Association of American Universities that aims to create a voluntary "framework" for improving teaching practices that institutions can adapt to their own situation and implement at their own pace.

"I'm very supportive of improving undergraduate STEM teaching," says Francis Collins, director of the National Institutes

"There's an entire industry devoted to measuring how important my research is, with impact factors of papers and so on. Yet, we don't even collect data on how I am teaching."

—Carl Wieman

overturning an academic culture that values research over teaching. Working in the White House Office of Science and Technology Policy (OSTP) as associate director for science, Wieman was the de facto science education czar for the Obama administration. But his 20 months on the job taught him just how hard it is to change prevailing attitudes within U.S. higher education.

While at OSTP, Wieman floated the idea of requiring universities to collect and disseminate information on their teaching practices to remain eligible for federal research dollars. The policy would be a stick to get universities to pay more attention to teaching, he reasoned.

"There's an entire industry devoted to measuring how important my research is, with impact factors of papers and so on," Wieman says. "Yet, we don't even collect data on how I am teaching. It receives no attention. ... If everything about teaching remains hidden, then universities can avoid having to devote anything more than minimal effort to doing it well. They can instead spend most of their

of Health (NIH), which spends more money on academic research than any other federal agency. "But this struck people as the wrong pathway by which to achieve the desired outcome, and not very fair."

As if taking on the nation's research establishment wasn't enough of a challenge, last June, Wieman received a sudden diagnosis of multiple myeloma. To deal with this health crisis, he abruptly resigned from his White House post and enrolled in a clinical trial at NIH using two experimental drugs. That treatment ended in January, and the 62-year-old Wieman says he's "happy and healthy."

Wieman, who is leaving UBC but declined to say where he's going, has returned to the lecture circuit with an updated version of his standard talk, entitled "Taking a Scientific Approach to Science and Engineering Education." He's definitely not

cowed by the prospect of taking a long, hard road toward his goal. In fact, his personal metric for any reform worth attempting is his ability "to generate significant opposi-

Online

sciencemag.org

S Podcast interview with author Jeffrey Mervis (http://scim.ag/pod_6130).

tion.” Speaking at a session of the February annual meeting of AAAS (which publishes *Science*) in Boston, Wieman said that transforming undergraduate teaching “passes that litmus test.”

Giving reform a chance

Wieman’s personality and upbringing seem well-suited to a grand challenge like remaking undergraduate science education. Before deciding on a scientific career, he embraced a succession of passions, including chess and tennis, which for a time were all-consuming. “Monomaniacal pretty much describes me,” Wieman confessed during a 2007 interview with the Nobel committee. “My view of everything is that you become good at something by focusing and working hard at it.” Eventually, he recalls, “science [became] such an activity.”

That doggedness served him well in pursuing his Nobel Prize-winning research. In 1925, Albert Einstein, building on the work of Indian physicist Satyendra Nath Bose, deduced that cooling a gas of certain atoms should make all the atoms suddenly flop into the same lowest energy quantum wave. Such a macroscopic matter wave is known as a Bose-Einstein condensate. Some 70 years later, Wieman and Eric Cornell of JILA, a lab run jointly by the National Institute of Standards and Technology and the University of Colorado, Boulder, achieved one by employing magnets and lasers to cool rubidium-87 atoms to within a millionth of a degree of absolute zero. In 2001, the two physicists shared the Nobel Prize in physics with Wolfgang Ketterle of the Massachusetts Institute of Technology in Cambridge, who achieved a similar result with sodium-23 atoms.

Wieman and Cornell at least had the advantage of knowing what a Bose-Einstein condensate would look like before they created one. In contrast, many faculty members might not recognize high-quality, student-centered learning because they may never have experienced it. Wieman admits that many notable scientists have thrived on a diet of traditional teaching practices and that the current rewards system at most universities gives faculty members little reason to try something different.

“I’m certainly not one to dismiss the importance of research,” Wieman says. “But people need to recognize how totally dominant the reward system is. There are a lot of faculty who feel, completely appropriately, that ‘I could spend more time improving my

teaching, but that’s not what I’m supposed to be doing.’ So you have to figure out a way for them to be able to improve their teaching without making a big sacrifice in their research activities.”

Wieman embarked on his quest to improve undergraduate education after pondering his own career as a professor and educator. And like the scientist that he is, he began by asking himself some basic questions. Why, he wondered, did students in his introductory courses do so poorly, and even regress, after he delivered lectures covering what they needed to know? Why couldn’t he identify at the outset which graduate students were most likely to succeed? And why did most of them become productive scientists after a few years in his lab?

Digging into the literature on teaching and learning yielded some insights. His graduate students had learned to think like scientists, he realized, by doing real science under the supervision of a world-class scientist. Developing expertise, he came to

“I know that Carl is skeptical
universities will do it on
their own. But I have yet
to be convinced that
they won’t.”

—Francis Collins, NIH director

understand, is a slow and arduous process marked by repeated failures.

“The apprentice model works pretty well in graduate school because the faculty member can see if the student is learning how to build a laser system, or write a paper, or give a professional talk,” says University of Colorado, Boulder, physicist and education researcher Noah Finkelstein, who has worked closely with Wieman and now directs the university’s newly formed Center for STEM Learning. “Those are things we actually want them to do. We give them feedback along the way, and we take in feedback from them and adjust our mentoring. But that system is just too costly at the undergraduate level.”

Instead, faculty members must interact

with hundreds of students in a large hall. Most choose to do that via a lecture. But research has shown that most students cling to their misconceptions even after sitting through a brilliant lecture.

What works better than lectures and homework problems, according to numerous studies, is having students work in small teams with instructors who can help them apply those basic concepts to real-life situations. But what’s the best way to implement active, student-centered learning? The answer, Wieman decided, lay in melding it with the concept of deliberate practice.

That idea, developed by psychologist K. Anders Ericsson of Florida State University in Tallahassee, treats the brain as a muscle that must be exercised to perform at its peak. It’s how a novice becomes an expert, whether in music, sports, or science. “We have learned that complex expertise is a matter not of filling up an existing brain with knowledge, but of brain development,” Wieman says.

Deliberate practice, Wieman wrote in the fall 2012 issue of *Issues in Science and Technology*, “involves the learner solving a set of tasks or problems that are challenging but doable and that involve explicitly practicing the appropriate expert thinking and performance.” The teacher, or coach, offers appropriate incentives to encourage students to master the necessary skills, as well as continuous feedback to help them remain on task. As with any sport, he notes, “[t]housands of hours of deliberate practice are typically required to reach an elite level of performance.”

The two concepts created an intellectual framework around which to transform undergraduate science. “Just as we have physics principles, here are the principles that work, and they are consistent with what others had done,” Wieman says. “It also allows you to go into disciplines where there hadn’t been much work done, like oceanography, and make some generalizations. It’s very much like science itself.”

In a 2011 paper in *Science*, Wieman and his colleagues describe the power of active learning and deliberate practice. The instructor for one section of an introductory physics class for engineers at UBC used these principles, while the other instructor delivered the normal lectures. The first group of students scored more than twice as high on a multiple-choice test of the material covered than did those in the control group.

“The results were so dramatic from this relatively modest experiment that the entire [physics] department had an epiphany,” remarks Simon Peacock, UBC’s dean of sciences. “It sent them a clear message: Wow, we can actually teach better.”

Wieman says that active learning and deliberate practice is now the norm in 99 UBC courses enrolling 31,200 students. Many are introductory courses taken by freshmen and sophomores who are still uncertain of their major field of study. “We have substantially changed more than half of the math and science courses a UBC student in the college of science will take in their first 2 years,” Wieman says, citing results from a recent survey of how faculty members have changed their teaching practices since the Carl Wieman Science Education Initiative was launched in 2007.

“We’ve hit it out of the park with earth and ocean sciences,” one of seven departments that are part of the university-funded initiative, Peacock says. “I will declare them to be a success.”

Wieman believes that deliberate practice can also help students in primary and secondary school who, for whatever reason, are ill-prepared for success in STEM subjects. His efforts have helped resolve “a huge controversy,” says NSF’s Singer, over whether the vast majority of students are capable of doing high-level math and science.

“Having Carl stand up and say we should stop doing STEM talent selection and start doing STEM talent development completely changes the nature of the conversation,” says Singer, on leave from her post as a biology professor at Carleton College in Northfield, Minnesota. “It’s really a question of how you structure the learning environment. And his work has shown that active learning strategies are more effective.”

From my way to the right way

What does it take to transform an undergraduate science course? Wieman’s approach relies heavily on a cadre of science teaching and learning fellows, who are typically post-docs. At its height, the Colorado initiative employed a dozen such fellows; at UBC, the number peaked at nearly two dozen.

The fellows are trained in the many steps needed to transform a university lecture course—steps that faculty members are unlikely to take on their own, either out of ignorance or because they simply don’t have the time to do what’s needed. Katherine

Perkins, who directs both the science education initiative at Colorado and the related PhET project (phet.colorado.edu), which has created thousands of research-based simulations of physical phenomena, calls the teaching and learning fellows “engines of change.”

Meeting for the first time with a faculty member, a fellow might start by asking what

courses are “owned” by the department and a consensus exists on what students are expected to know regardless of who is teaching the course.

A transformed course typically begins not with a lecture but with a clicker question. Students gather in small groups to discuss it, and a fellow assigned to the course circulates through the classroom to

“Having Carl stand up and say we should stop doing STEM talent selection and start doing STEM talent development completely changes the nature of the conversation.”

—Susan Singer, head of NSF undergraduate education

the faculty member wants students to know how to do at the end of the course. That’s a more useful metric than asking what a student “should understand,” explains Beth Simon, director of the Center for Teaching Development at the University of California, San Diego, who spent the 2007 to 2008 academic year at UBC as a fellow in the computer science department before returning to UCSD.

Once the faculty member articulates the real goals of the course, those skills are converted into learning objectives. The next step is to write up multiple-choice questions aimed at helping students achieve each learning objective. The so-called clicker questions (the name comes from the electronic device that students use to record their answers) usually focus on common student misconceptions about the concepts.

The questions become the basic curriculum for the course. But getting from skills to clicker questions can be difficult. Simon figured that the final exam would provide a useful guide to what students were expected to learn. Instead, instructors would admit that they didn’t really know what concepts some test questions were meant to measure, she says, and that other questions covered concepts not central to the course.

Most courses come with only a three- or four-sentence description in the syllabus. That brevity gives whoever is teaching the course a lot of leeway. Some faculty members have been teaching the same course for years, Simon says, and for them, “learning outcomes were a nonstarter. ‘I teach 101 my way,’ they would say.” In contrast, some

guide the inquiry process. Once the students have punched in their answers, the faculty member might offer a microlecture aimed at correcting their mistakes and filling in gaps in their knowledge. Once the concept is clear, the class moves on to the next clicker question.

Students taking transformed courses are usually more active than in a typical lecture class. Faculty members need to remind students regularly why they will not be lecturing, Simon says, as well as explain the importance of peer instruction. To get the most from the class time, students are assigned outside reading and turn in homework that measures their understanding of the material.

Some students are uncomfortable with this approach—even if it’s more effective. “I remember getting an evaluation from one [UCSD] student who had just finished my course,” says Simon, a pioneer in the use of peer instruction within her field. “I loved it. It read, ‘I just wish she’d have lectured. Instead, I had to learn the material myself.’”

The increased student engagement in a transformed course is music to the ears of the average faculty member. “Most faculty want their students to learn more,” says Perkins, whom Wieman hired in 2003 as one of the initiative’s first teaching fellows. “They look at the final exam, sigh, and say, ‘Why did only 60% get that question right?’” Simon adds, “If they can have more fun, they will choose to use these methods.”

A department should plan on spending about 5% of its budget for 5 years to transform its courses, Wieman says. Lesser

amounts are required to sustain progress, he adds, although new faculty members must be trained and existing faculty members need ongoing support and, occasionally, a sympathetic ear. At Colorado, for example, departments competed for grants of roughly \$600,000 to \$800,000 each. UBC's \$10 million commitment to the initiative allowed Wieman to double the size of departmental awards, and a more recent \$2 million donation from David Cheriton, a professor of computer science at Stanford University, is fueling reform within the math and computer science department.

Wieman's campaign to transform departments isn't the only game in town. Finkelstein's new center at Colorado, funded by an NSF planning grant, is supposed to serve as a focal point for some 75 STEM-related activities on campus. And Colorado's Perkins hopes that NSF will put up several

"I'm more optimistic than I was a year ago," he adds, "because people who we thought weren't interested are now saying, 'Look, I made this change and I'm thinking of doing more.' But I won't give you good odds that they will still be doing it in 10 years."

Carrot or stick?

In 2010, Wieman decided to come to Washington for the chance to influence undergraduate science education on a national scale. "My top priority at OSTP was to improve undergraduate education," he says. "We know what to do that will help students learn more and be more successful and how to get a broader group of students doing it."

While there, Wieman came up with his simple, market-driven first step: Require universities to compile and release data on

and he says that none of the community's objections are valid.

For starters, he says, colleagues at UBC and Colorado have created a questionnaire that collects such data and requires only a few hours of effort by an entire department—"a tiny amount compared to what is spent in a single faculty meeting," he snickers. Universities have no incentive to game the system, he adds, because students would soon expose any institution that had submitted bogus information. And he scoffs at the idea that tracking a fundamental purpose of a university could be regarded as a "burden."

Part of their objections, he speculates, is that the data could prove embarrassing. "Educational transparency is a threat to their status," he argues. "Maybe it won't make them look so good."

NIH's Collins says that's not the reason he prefers a voluntary approach. "I know that Carl is skeptical universities will do it on their own," he says. "But I have yet to be convinced that they won't. I don't know that all universities will want to participate. But I think there will be some who would say, 'Yeah, we believe in this. It's the right thing to do.'"

Government officials and university leaders typically defend the value of federally funded research by citing its role as an engine of economic growth. In the case of biomedical research, they also note its potential to save lives. But Wieman doesn't think those arguments really address the growing clamor from the public and politicians for universities to show that an undergraduate education is worth the rising cost of tuition. That skepticism, he says, has also fueled a decadelong assault by many state legislatures on their flagship public universities.

A more effective response, Wieman says, would be for university presidents to emphasize how research can lead to better teaching. "I think the solution is to show that you can really use that research expertise to improve education," he says. "Deliberate practice and other approaches is calling on, and demanding of, the research expertise embodied by that faculty."

"If you pitch that message," he continues, "then suddenly it becomes clear how having a great research university translates into better education for students in my state. Right now it's not worth the investment, because it's not happening. But it could."

—JEFFREY MERVIS

"There are a lot of faculty who feel, completely appropriately, that 'I could spend more time improving my teaching, but that's not what I'm supposed to be doing.'"

—Carl Wieman

million dollars for a Web site to help faculty members use the PhET simulations that she and others have created and to study their impact on teaching and learning.

But money remains tight. Wieman says he can't afford to conduct the rigorous, outside assessments that normally accompany NSF-funded reforms because he feels that institutional funds should redound to the benefit of the institution. However, the dearth of peer-reviewed publications has led some scientists to question what Wieman's Colorado and UBC initiatives have accomplished.

"When people ask what we've done," Finkelstein says, "and I say we've shifted institutional identity and culture, half the time their response is, 'Wow, that's terrific.' But the other half say, 'So all you've done is talk?'"

Wieman himself offers a frank answer when asked whether he expects the UBC reforms to stick. "That's why you do research," he says. "This was a one-time intervention. And people have a right to wonder what will happen next.

their teaching methods as a condition for receiving federal research funds. As students began using the data released by universities to help choose a college, he reasoned, universities would feel compelled to improve their teaching practices in order to attract the best applicants. "If an agency were to require every grantee to provide this information," he says, "then the next year teaching would look completely different because somebody is looking at it."

Wieman promoted the idea tirelessly in meetings with his government colleagues as well as the presidents of several leading research universities, seeing it as a painless way to propel reform. But they pushed back hard. It's hard to define particular teaching practices, they told Wieman. Self-reported data are unreliable, they added, and collecting such data would be a burden. Last April, the presidents of several prominent universities even wrote a letter to then-White House Chief of Staff Jacob Lew in an attempt to head off Wieman's proposal.

A few months later, Wieman was gone. But he hasn't changed his mind one iota,

REVIEW

The Challenge of Education and Learning in the Developing World

Michael Kremer,¹ Conner Brannen,¹ Rachel Glennerster^{2*}

Across many different contexts, randomized evaluations find that school participation is sensitive to costs: Reducing out-of-pocket costs, merit scholarships, and conditional cash transfers all increase schooling. Addressing child health and providing information on how earnings rise with education can increase schooling even more cost-effectively. However, among those in school, test scores are remarkably low and unresponsive to more-of-the-same inputs, such as hiring additional teachers, buying more textbooks, or providing flexible grants. In contrast, pedagogical reforms that match teaching to students' learning levels are highly cost effective at increasing learning, as are reforms that improve accountability and incentives, such as local hiring of teachers on short-term contracts. Technology could potentially improve pedagogy and accountability. Improving pre- and postprimary education are major future challenges.

Over the past two decades, a new wave of randomized evaluations has examined how developing countries can help (i) children who are not in school gain access to education and (ii) those in school improve their often abysmal learning levels. Randomized evaluations can help disentangle the causal impact of programs from mere correlations, but there has been debate about the extent to which results will generalize across different contexts. We review the evidence on program impact and present a new cost-effectiveness analysis. In most cases, we find a remarkable degree of consistency between the impacts of similar programs across different geographic areas. However, the impact of programs designed to improve teacher performance and school accountability seems more context-dependent and sensitive to small differences in program design.

Broadening Access to Education

Randomized evaluations consistently find that making school more financially attractive can increase school participation (1). Conditional cash transfer (CCT) programs provide financial support to poor mothers if their children obtain basic medical care and attend school regularly. Mexico's initial CCT program (PROGRESA) in 1998 increased girls' transition rate from elementary to junior secondary school by 14.8 percentage points and boys' by 6.5 percentage points (2). After the success of PROGRESA, more than 30 countries established similar programs. Many of these have been subject to randomized evaluations, yielding similar results (3).

Most CCT programs involve financial transfers that constitute a large fraction of income for poor households, but three studies show that even small transfers can have large effects. In Malawi, a monthly \$5 CCT was as effective as one of \$10 (4). In Kenya, providing a free school uniform (costing less than \$8) reduced dropout rates among girls by 3.1% from a base of 18.8% (5).

The finding that small short-run costs can substantially influence life decisions with important long-run consequences is consistent with insights from behavioral economics (6). Behavioral economics also explains why matching the timing of payments under CCTs to the timing of school fees can raise the cost-effectiveness of these programs (7). Merit scholarship programs can simultaneously increase access to schooling and stimulate learning by motivating students to work harder, do more homework, and attend school more often (8).

In some regions of the world, gender gaps in enrollment are large. The studies cited above find substantial effects of financial incentives on girls' school participation. Despite much discussion, there is limited rigorous evidence on whether latrines increase girls' school attendance by allowing them to manage menstruation. A randomized trial in Nepal found no impact of providing sanitary products on school participation (9).

Beyond price, distance to school is also important for school participation, especially if social norms or safety concerns make it difficult for girls to travel far from home. In Afghanistan, a program that encouraged communities to donate space in existing buildings to establish local schools increased enrollment of girls by 42 percentage points. Girls' primary school enrollment fell 19 percentage points and boys' 14 percentage points per additional mile to school (10).

Two approaches to increasing school participation are particularly cost-effective (11). School-based deworming cut student absenteeism by a quarter in a study in western Kenya (12), and students in neighboring schools also attended more because the intervention interrupted the disease transmission cycle. One hundred dollars invested in mass school-based deworming would generate 14 additional years of schooling. Follow-up results from Kenya, as well as nonrandomized evidence from deworming in the U.S. South, suggests that deworming generates large, long-run educational and income benefits (13, 14). Studies in the Dominican Republic and Madagascar found that simply informing those who underestimate the correlation between education and earnings about how average earnings vary with education can increase school participation at very low cost (15, 16).

Education Quality

Although the quantity of schooling has expanded rapidly, quality is often abysmal. In India, 31% of children in third grade could not recognize simple words (17). Nineteen percent of teachers were found absent in unannounced visits to a sample of schools in six countries (18).

Figure 1 presents new work comparing the cost-effectiveness of 30 primary school programs in raising test scores subject to randomized evaluation where study authors have made detailed cost information available. These comparisons should be considered as one input among others in assessing policy, especially because some approaches may create benefits beyond test scores and because a standard deviation of test scores may have different meanings in different contexts. Some interventions may be more applicable than others in two particular lotteries (11) (further comparisons, as well as the underlying calculations, will be made available at www.povertyactionlab.org/policy-lessons/education/student-learning). The left panel shows the estimated impacts measured in standard deviations (SDs) and the 90% confidence intervals. The right panel shows (on a log scale) how many SDs of test-score improvement could be gained from an investment of \$100. In some cases, costs would likely fall if programs were implemented at scale, but for consistency we show costs from the study. We show effects over different time horizons where examined.

Many programs that increased quantity of education also promoted learning. For example, the program in Afghanistan raised scores dramatically (10), deworming increased Kenyan girls' passing rate on primary-school exams by 25% (13), and the Madagascar program increased test scores by 118 SDs for every \$100 spent (16).

Despite very low levels of resources, providing more-of-the-same educational inputs without changing pedagogy or accountability typically has very limited impacts on test scores. For example,

¹Harvard University, Cambridge, MA 02138, USA. ²Abdul Latif Jameel Poverty Action Lab, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

*Corresponding author. E-mail: rglenner@mit.edu

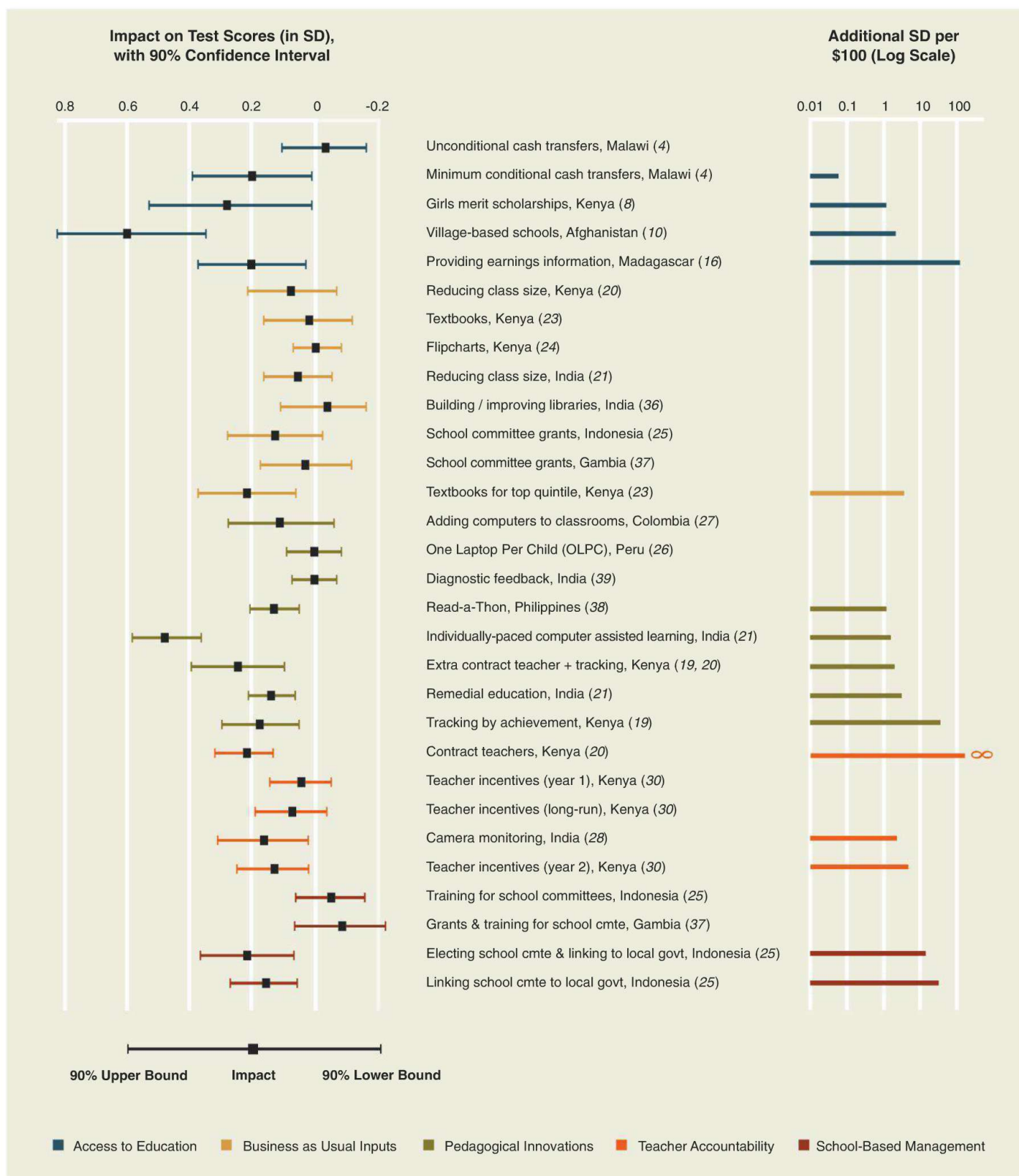


Fig. 1. Impacts on test scores from randomized trials of primary school programs in the developing world. Results drawn from 18 randomized studies that report test-score outcomes. The charts also rely on detailed cost data provided

by the authors of these studies and discussions with them about the most appropriate way to calculate cost effectiveness. The underlying data and calculations are available at www.povertyactionlab.org/policy-lessons/education/student-learning.

when a Kenyan program brought in local teachers, roughly halving class size (from a base of 80) test scores did not improve significantly for students remaining with the government teacher (19, 20). A study in two Indian states found similar results (21). In contrast, a U.S. randomized evaluation found reducing class sizes improved test scores (22). One hypothesis is that extra resources have more impact when school accountability is stronger.

Evidence on nonteacher inputs is also discouraging. Neither providing additional textbooks nor supplying instructional flip charts increased test scores in Kenya (23, 24). Providing grants to local school committees to support their schools did not improve test scores in Indonesia (25).

Some input studies provide clues to why test scores did not increase. Although provision of government-sanctioned textbooks in Kenya had no impact on the average student, it did improve learning for those that started the year with higher learning levels (23). This result suggests that the textbooks were tailored to the best performing children. This study led to a series of randomized evaluations examining the effectiveness of adjusting curricula to the child's level.

In many developing countries, educational systems and curricula focus on children with the highest learning levels. Several different pedagogical strategies designed to match the level of instruction to the child's level proved cost-effective at improving learning. A remedial education program in India generated a 3.01-SD test-score gain per \$100 spent (21). A Kenyan program hired extra teachers, divided grade 1 classes into two, and compared learning when students were assigned to classes on the basis of initial

preparedness or random assignment. Tracking by initial level of preparedness increased test scores at both higher and lower levels of initial preparedness. Technology can be used to tailor learning to a student: A computer-assisted learning (CAL) program in India, which used math software that allowed children to learn at their own pace, increased scores by 0.48 SD (21), or 1.54 SD per \$100 spent. But there were no significant test-score gains in Peru from the One Laptop Per Child program or a Colombian CAL program (26, 27), potentially because the programs did not tailor instruction to each student's level of knowledge and because the computers in Colombia were not tied to the curriculum and therefore not widely used.

Evidence from India suggests that incentivizing teacher presence in class can improve test scores (28). However, the effectiveness of several approaches to improving teacher incentives seems to differ across studies. Tying teacher pay to student test scores improved learning in India (29), but in Kenya teachers responded primarily by teaching to the test (30). Some argue that informing parents about school conditions can lead them to demand better services from public schools. An evaluation of this approach in India found no impact (31). In contrast, a program in Pakistan found providing parents with information on both public and private (nonelite) schools caused low-performing schools to improve their performance and higher-performing private schools to reduce their fees (32). An important topic for future work is whether these contrasting results are due to differences in the details of the program or differences in context.

Programs that go further in empowering, rather than just informing, local communities seem more successful. Introducing local school com-

mittees does not in itself seem to improve performance, nor does training these committees (25) or informing members of their duties and levers for improving quality (31). However, giving school committees more formal links to government and making them more representative through elections did improve test scores in Indonesia (25).

Studies in both Kenya and India found test-score gains from supplementing civil-service teachers with locally hired teachers hired on short-term contracts (20, 21). The Kenya study suggests that if the government planned on hiring enough teachers to have a class size of 40 students in first grade, initially hiring teachers locally on short-term contracts at about one-fourth the salary, would in theory be infinitely cost-effective: It would improve learning while reducing costs relative to the current system. Another study in Kenya highlighted the pressures governments may face to turn locally hired contract teachers into civil servants—a move that can undermine their effectiveness (33).

A more radical step would be to provide parents with vouchers and allow them to choose private schools for their children. A program in Colombia that combined elements of vouchers and merit scholarships (vouchers were contingent on grade completion) led to higher rates of secondary school completion (34). More evidence from other developing country contexts would be very useful. Evidence on the overall test-score impact of vouchers in the United States is mixed, but there is at least some evidence that disadvantaged African-American populations gain (35). One hypothesis is that these programs have a bigger impact where governance problems in public schools are more severe.

We have learned a great deal about how to help those children who are not in school obtain access to schooling, and a number of general themes have emerged from research on how to ensure children in primary school are learning. Providing additional inputs without changing pedagogy or governance has had limited impact, whereas adapting teaching methods to reach the varied learning levels in developing countries is highly effective. There are three major challenges for education in developing countries, including using technology to improve pedagogy and school accountability, cost-effectively improving education access and quality outside primary school, and understanding the role of private education. In all of these areas, it will be important to study long-term impacts on learning, noncognitive skills, labor market outcomes, health and family outcomes, and civic attitudes and behavior. We have much more to learn.

Grand Challenges

Use technology to improve pedagogy, management, and accountability. Most developing-country classrooms contain a dramatic range of learning levels. Programs that seek to tailor teaching to children's learning level have proved effective, and educational technology that tailors instruction to students' knowledge levels could play an important role. In addition, monitoring technologies could help improve accountability and facilitate better incentives in education systems where one in five teachers is absent.

Improve access to, and the quality of pre- and postprimary education. Substantial evidence exists on how to increase the quantity and quality of education in primary schools. But much more research is needed at other education levels. There is intriguing evidence that preprimary and early childhood programs could be highly effective in improving learning, but we need to know more. Secondary, vocational, and tertiary schooling are also important areas for research because of the rapid rise in attendance, high costs, concerns about curricular relevance, and the patchy state of current knowledge.

Develop appropriate policies for regulating and supporting the private sector in education. In large part because of the poor quality of government-run education, private schools are increasingly common, even for the poor. But many of these schools are of low quality, and we know little about how to improve their performance or the potential role of government in supporting and regulating them.

References and Notes

1. M. Kremer, A. Holla, *Annu. Rev. Econ.* **1**, 513 (2009).
2. T. P. Schultz, *J. Dev. Econ.* **74**, 199 (2004).

3. A. Fiszbein, N. Schady, F. H. Ferreira, *Conditional Cash Transfers: Reducing Present and Future Poverty* (World Bank, Washington, DC, 2009).
4. S. Baird, C. McIntosh, B. Ozler, "Cash or condition? Evidence from a cash transfer experiment" (World Bank Policy Research Working Paper Series 5259, World Bank, Washington, DC, 2010).
5. E. Duflo, P. Dupas, M. Kremer, "Education, HIV, and early fertility: Experimental evidence from Kenya" (MIT Working Paper, Massachusetts Institute of Technology, 2012), <http://economics.mit.edu/files/6951>.
6. M. Kremer, R. Glennerster, in *Handbook of Health Economics*, M. V. Pauly, T. G. McGuire, P. P. Barros, Eds. (Elsevier, North Holland, Netherlands, 2011), vol. 2, chap. 4.
7. F. Barrera-Osorio, M. Bertrand, L. L. Linden, F. Perez-Calle, *Am. Econ. J. App. Econ* **3**, 167 (2011).
8. M. Kremer, E. Miguel, R. Thornton, *Rev. Econ. Stat.* **91**, 437 (2009).
9. E. Oster, R. Thornton, *Am. Econ. J. App. Econ* **3**, 91 (2011).
10. D. Burde, L. Linden, "The effect of village-based schools: Evidence from a randomized controlled trial in Afghanistan" (NBER Working Paper No. 18039, National Bureau of Economic Research, Cambridge, MA, 2012).
11. I. Dhaliwal, E. Duflo, R. Glennerster, C. Tulloch, in *Education Policy in Developing Countries*, P. Glewwe, Ed. (Univ. of Chicago Press, Chicago, in press).
12. E. Miguel, M. Kremer, *Econometrica* **71**, 159 (2004).
13. S. Baird, J. Hamory Hicks, M. Kremer, E. Miguel, "Worms at work: Long-run impacts of hild health gains" (Harvard University Working Paper, Cambridge, MA, 2012), <http://scholar.harvard.edu/kremer/publications/worms-work-long-run-impacts-child-health-gains>.
14. H. Bleakley, *Q. J. Econ.* **122**, 73 (2007).
15. R. Jensen, *Q. J. Econ.* **125**, 515 (2010).
16. T. Nguyen, "Information, role models and perceived returns to education: Experimental evidence from Madagascar" (MIT Job Market Paper, Cambridge, MA, 2008); www.povertyactionlab.org/sites/default/files/documents/Nguyen%202008.pdf.
17. E. Pratham, L. R. Card, Annual Status of Education Report (2011), http://images2.asecentre.org/homepage/Conference_Pack/enrollment_and_learning.pdf.
18. N. Chaudhury, J. Hammer, M. Kremer, K. Muralidharan, F. H. Rogers, *J. Econ. Perspect.* **20**, 91 (2006).
19. E. Duflo, P. Dupas, M. Kremer, *Am. Econ. Rev.* **101**, 1739 (2011).
20. E. Duflo, P. Dupas, M. Kremer, "School governance, teacher incentives, and pupil-teacher ratios: Experimental evidence from Kenyan primary schools" (NBER Working Paper No. 14475, National Bureau of Economic Research, Cambridge, MA, 2012).
21. A. Banerjee, S. Cole, E. Duflo, L. Linden, *Q. J. Econ.* **122**, 1235 (2007).
22. A. Krueger, D. M. Whitmore, *Econ. J.* **111**, 1 (2001).
23. P. Glewwe, M. Kremer, S. Moulin, *Am. Econ. J. Applied Econ.* **1**, 112 (2009).
24. P. Glewwe, M. Kremer, S. Moulin, E. Zitzewitz, *J. Dev. Econ.* **74**, 251 (2004).
25. M. Pradhan *et al.*, "Improving educational quality through enhancing community participation: Results from a randomised field experiment in Indonesia" (World Bank Policy Research Working Paper 5795, World Bank, Washington, DC, 2011).
26. J. Cristia, P. Ibarra, S. Cueto, A. Santiago, E. Severin, "Technology and child development: Evidence from the One Laptop per Child program" (IZA Discussion Paper No. 6401, Forschungsinstitut zur Zukunft der Arbeit GmbH [IZA], Bonn, Germany, 2012).
27. F. Barrera-Osorio, L. Linden, "The use and misuse of computers in education: Evidence from a randomized controlled trial of a language arts program" (working paper, 2009), www.leighlinden.com/Barrera-Linden%20Computadores_2009-03-25.pdf.
28. E. Duflo, R. Hanna, S. P. Ryan, *Am. Econ. Rev.* **102**, 1241 (2012).
29. K. Muralidharan, V. Sundararaman, "Teacher performance pay: Experimental evidence from India" (NBER Working Paper 15323, National Bureau of Economic Research, Cambridge, MA, 2009).
30. P. Glewwe, N. Ilias, M. Kremer, *Am. Econ. J. App. Econ* **2**, 205 (2010).
31. A. Banerjee, R. Banerji, E. Duflo, R. Glennerster, S. Khemani, *Am. Econ. J. Econ. Pol.* **2**, 1 (2010).
32. T. Andrabi, J. Das, A. Khwaja, T. Vishwanath, T. Zajonc, "Learning and Educational Achievements in Punjab Schools (LEAPS): Insights to inform the education policy debate" (LEAPS Report, 2007), http://leapsproject.org/assets/publications/LEAPS_report.pdf.
33. T. Bold, M. Kimenyi, G. Mwabu, A. Ng'ang'a, J. Sandefur, "Interventions & Institutions Experimental Evidence on Scaling up Education Reforms in Kenya" (preliminary draft, 2012), http://www.cgdev.org/doc/kenya_rct_webdraft.pdf.
34. J. Angrist, E. Bettinger, E. Bloom, E. King, M. Kremer, *Am. Econ. Rev.* **92**, 1535 (2002).
35. C. Rouse, *Q. J. Econ.* **113**, 553 (1998).
36. E. Borkum, F. He, L. Linden, "School libraries and language skills in Indian primary schools: A randomized evaluation of the Askshara library program" (NBER Working Paper No. 18183, National Bureau of Economic Research, Cambridge, MA, 2009).
37. M. Blimp, D. Evans, "School-based management and educational outcomes: Lessons from a randomized field experiment" (Stanford University Working Paper, Stanford, CA, 2011), www.stanford.edu/~mpblimp/BlimpoEvans2011.pdf.
38. A. B. Abeberese, T. Kumler, L. Linden, "Improving reading skills by encouraging children to read: A randomized evaluation of the Sa Aklat Sisikat reading program in the Philippines" (NBER Working Paper No. 17185, National Bureau of Economic Research, Cambridge, MA, 2012).
39. K. Muralidharan, V. Sundararaman, *Econ. J.* **120**, F187 (2010).

Acknowledgments: We are extremely grateful to the authors of the studies included in the cost-effectiveness analysis for providing detailed data on the program costs and discussions on ways to most appropriately calculate cost-effectiveness, the J-PAL policy team, three anonymous referees, and B. Nordgren for designing Fig. 1.

10.1126/science.1235350

REVIEW

Understanding Neurocognitive Developmental Disorders Can Improve Education for All

Brian Butterworth^{1,2,3*} and Yulia Kovas^{3,4,5}

Specific learning disabilities (SLDs) are estimated to affect up to 10% of the population, and they co-occur far more often than would be expected, given their prevalences. We need to understand the complex etiology of SLDs and their co-occurrences in order to underpin the training of teachers, school psychologists, and clinicians, so that they can reliably recognize SLDs and optimize the learning contexts for individual learners.

In the not-too-distant past, children who were unable to learn the usual school subjects to a normal level were classified as having mental retardation, or what we would now call "intellectual disability" (U.S.) or "learning disability"

(UK). These labels are still sometimes applied to children with severe delays in learning to read and spell, whom we would now call dyslexic, or those with serious social difficulties, whom we would now call autistic (1).

Extensive research in cognitive development shows that children with normal or even superior IQs, and who clearly are not mentally retarded, can fail to reach acceptable standards in key curriculum areas, such as literacy (2) and numeracy (3). The terms intellectual or learning disability are currently reserved for those whose score on an IQ test is below 70 (the lowest 2%, approximately).

The evidence outlined in this Review presents multiple reasons why it is difficult to define neurocognitive developmental disorders. Complex genetic, brain, and cognitive processes underlying these conditions remain poorly understood.

¹Institute of Cognitive Neuroscience, University College London, Alexandra House, 17 Queen Square, London WC1N 3AR, UK.

²IRCCS Ospedale San Camillo, Venice, Italy. ³Melbourne School of Psychological Sciences, University of Melbourne, Melbourne, Australia. ⁴Department of Psychology, Tomsk State University, Tomsk, Russia. ⁵Department of Psychology, Goldsmiths, University of London, London, UK. ⁶Social, Genetic and Developmental Psychiatry Centre, King's College London, London, UK.

*Corresponding author. E-mail: b.butterworth@ucl.ac.uk

Throughout the Review, we refer to specific learning disabilities (SLDs), following the U.S. federal law definition of “a disorder in one or more of the basic psychological processes involved in understanding or in using language, spoken or written, that may manifest itself in an imperfect ability to listen, think, speak, read, write, spell, or to do mathematical calculations” (4). We apply this term to such diverse conditions as dyslexia or autistic spectrum disorder to emphasize the uncertainty about their classifications. Irrespective of definitions, SLDs are thought to affect approximately 10% of the population (Table 1) and have a profound effect on educational outcomes. Unlike learners with intellectual disability, who need at least some educational support in all curriculum areas and, in severe cases, support in daily living, those with SLDs need support mainly in those areas of specific weakness. Here we focus on just five SLDs (Table 2).

The Co-Occurrence of Specific Learning Disabilities

An additional problem for the educator is that SLDs co-occur far more often than would be expected given their prevalences (Table 1). If, for example, dyslexia and dyscalculia were entirely independent conditions, then the expected rate of co-occurrence would be the product of the base rates: i.e., 7% × 6%, or about 0.5%. However, one population-based study with these prevalences found that 23 to 49% of children in grades 2 to 4 had disabilities of both literacy and numeracy (5). Studies of selected samples of other SLDs also indicate a higher level of co-occurrence than that expected by chance. For example, of children with attention-deficit/hyperactivity disorder, 33 to 45% also suffer from dyslexia (6); and 11% from dyscalculia (7).

The co-occurrence of autism spectrum disorder and attention-deficit/hyperactivity disorder have also been reported, although not consistently across the age range (8); the occurrence of numeracy and literacy disorders in autistic spectrum disorder is roughly equivalent to that in typically developing learners (9).

Domain-General Explanations

Many studies have sought to explain SLDs and their co-occurrence in terms of “domain-general” cognitive capacities such as those measured by IQ tests, tests of working memory (the retention of task-relevant information for the duration of the task), or tests of processing speed. However, there are important differences in what the commonly used tests measure. For example, the widely used Wechsler IQ tests for adults and children (10, 11) require knowledge of vocabulary, numbers, and arithmetic, whereas other tests require only spatial and reasoning skills (12). Different intelligence tests may thus give different assessments. Nevertheless, many authorities, including the U.S. Office of Educa-

tion and the American Psychiatric Association, recommend using a significant discrepancy between IQ and a test of reading, mathematics, or social ability as the criterion for diagnosis of a SLD (see Table 2 for examples). This makes the diagnosis more probable for individuals of high intelligence and excludes the possibility that an individual can have both low intelligence and an SLD. In fact, SLDs may actually cause poor performance on some IQ tests. This is most obvious where the IQ test depends on reading and understanding and also a reasonable degree of numeracy (13).

Measures of working memory include tests of the ability to reproduce a string of digits in the presented order, whereas other tasks tap the ability to modify the contents of memory in response to current task demands. The association between working memory and SLD depends on the working memory task used and the SLD being assessed (3, 14).

Core Cognitive Deficits

Given the problems with trying to explain the varieties of SLDs in terms of domain-general capacities, much research has been motivated by the postulation of core cognitive deficits that can give rise to the observed behavior. Core deficits

themselves can have many causes and variable behavioral manifestations (Fig. 1).

Core Deficit in Dyslexia

At the cognitive level, a large majority of dyslexic children seem to suffer from a phonological deficit: a deficit in some aspects of the processing of speech sounds and their mental representation, although subtle visual or attention difficulties may contribute in some cases (15). Dyslexic symptoms depend on the regularity of the mapping between letters and sounds in alphabetic orthographies and thus will present in different ways in, for example, Italian and English, but its neurological basis is always found in areas of the brain that link letters to speech sounds (16).

Core Deficit in Dyscalculia

Children with dyscalculia show a core deficit in processing numerosities, which is revealed in slower and less accurate enumeration of small sets of objects and in comparing the numerosities of sets of objects or the magnitude of digits (17). However, good language abilities appear to be needed for the typical development of counting, calculation, and arithmetical principles (18).

Table 1. Estimated prevalences of five specific learning difficulties. NIH research funding for these SLDs in 2000–2009 varied widely (1, 32)

SLD	Estimated prevalence (%)	NIH research funding in U.S. \$1000s
Dyslexia	4–8	27,283
Dyscalculia	3.5–6.5	1,574
Attention-deficit/hyperactivity disorder	3–6	532,800
Autism spectrum disorder	1	851,270
Specific language impairment	7	28,611

Table 2. Definitions of SLDs. Adapted from (19, 20). See the text for more detailed characterization of these deficits.

SLD	Definitions
Development dyslexia	Developmental disorder in learning to read, not due to impairments in general intelligence, sensory problems, emotional disturbances, or inadequate schooling.
Developmental dyscalculia	Substantial underachievement on a standardized test of arithmetic relative to the level expected, given age, education, and intelligence, which causes disruption to academic achievement or daily living.
Attention-deficit/hyperactivity disorder	Symptoms of inattention, hyperactivity, and impulsivity, such that these symptoms cause clinically significant distress or impairment in social, academic, or occupational functioning.
Autism spectrum disorder	Impairments of social interaction and communication and repetitive, stereotyped behavior.
Specific language impairment	Significant deficits in expressive or receptive language, not due to sensory or environmental deprivation, co-occur with nonverbal intelligence within the average range

Core Deficit in Attention-Deficit/Hyperactivity Disorder

Children diagnosed with attention-deficit/hyperactivity disorder may have several core cognitive deficits, including one in attention and one in controlling, and especially inhibiting, behavior. The American Psychiatric Association distinguishes two subtypes, inattentive and hyperactive-compulsive (19), whereas the World Health Organization distinguishes a hyperkinetic disorder subtype from a comorbid conduct disorder subtype (20).

Core Deficit in Autism Spectrum Disorder

A core deficit in representing one's own and other people's thoughts and feelings is implicated in this condition, which is sometimes called a deficit in the theory of mind (21). As a consequence, individuals with autism have impairments in communication that depends on understanding others' intentions. However, the nonsocial aspects of autism, such as obsession with detail, are not explained by this deficit alone (22).

Core Deficit in Specific Language Impairment

Children diagnosed with this condition have difficulties with the meaning of words, syntax, and pragmatics, despite adequate intelligence, sensory apparatus, and exposure to language input. Specific language impairment appears to result from several core deficits, including the phonological deficit that is shared with dyslexia. The vast majority of children with this condition perform at least 1 SD below age controls, not only on comprehending texts, which will depend on understanding word meanings, grammar, and pragmatics, but also on reading aloud single words, which depends largely on the ability to map from letters onto the component sounds of words (13).

More generally, although core deficits frequently co-occur, they do not appear to interact. For example, children with attention-deficit/hyperactivity disorder alone performed relatively poorly on tasks requiring sustained attention, whereas children with dyslexia performed more poorly on phonological tasks; however, children with both conditions were not worse on either task (23). Similarly, children with both dyscalculia and dyslexia were no worse on tests of numerosity processing and phonological processing than those with just one condition (3, 24).

Neurological Basis of SLDs

The representative but not exhaustive evidence presented in Table 3 suggests that each SLD is associated with an abnormality in a distinct neural network. The neuroanatomical differences between learners with SLDs and typically developing learners usually have been found in magnetic resonance imaging (MRI) studies. However, it should be noted that important differ-

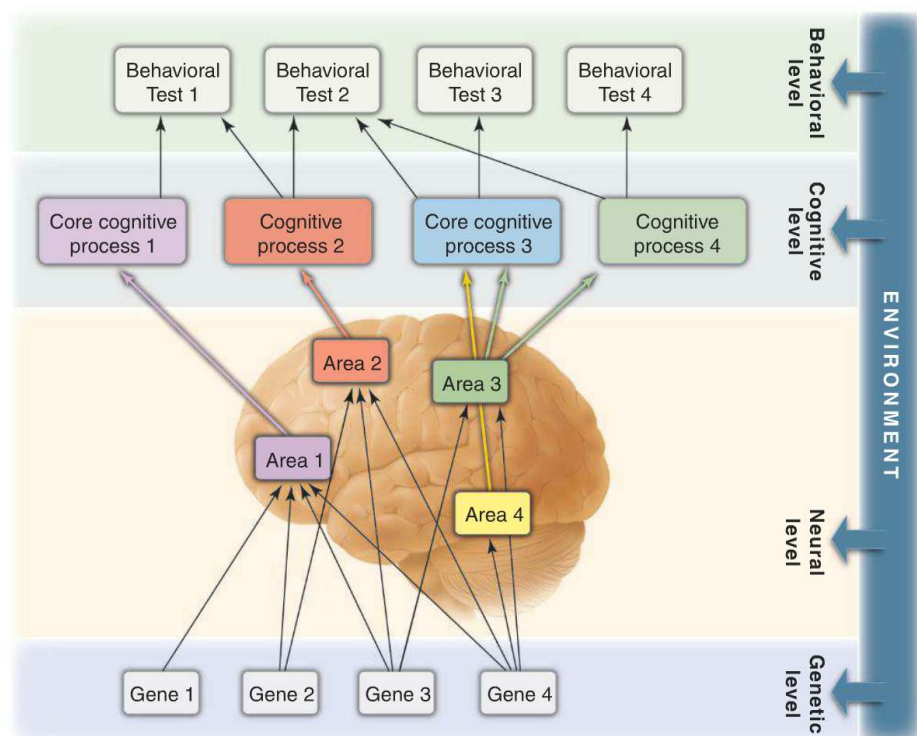


Fig. 1. Networks of interaction. Schematic model of the relationships among levels of explanation—genetic, neural, cognitive, and behavioral—following the causal modeling framework (34). There can be many-one, one-many, many-many, and one-one relationships between levels. A domain-general cognitive process and a domain-specific core cognitive process can have effects on more than one behavioral test, and performance on a behavioral test may be affected by more than one cognitive process. Moreover, one cognitive process may depend on another (e.g., memory on attention), and one behavior may causally affect another (e.g., poor reading may impair mathematical problem solving).

Table 3. Typical results for structural brain imaging in which probands with an SLD differ significantly from typically developing controls.

	Structural difference with controls	Subjects
Dyslexia (39)	Decreased gray matter density in left midtemporal gyrus and increased density in mid-posttemporal gyrus Decreased white matter volume in left arcuate fasciculus	10 Italian, 11 French, 11 English probands 9 Italian, 12 French, 11 English controls
Dyscalculia (40)	Decreased gray matter density in left intraparietal sulcus	12 probands, 12 controls
Attention-deficit/hyperactivity disorder (41, 42)	1. Decreased overall brain volume and cortical thickness 2. Decreased volume of anterior cingulate cortex 3. Decreased volume of frontal cortex 4. Cerebellum	1. 59 probands, 80 controls. 2. to 4. Review and meta-analysis
Autism spectrum disorder (43)	Greater total brain volume and grey matter volume throughout life span, most prominently in frontal lobe Greater prepuberty white matter volume	Review and meta-analysis
Specific language impairment (44)	Abnormal perisylvian asymmetry	20 probands, 12 controls

ences may not show up in the structure or activity of the brain as revealed by MRI, such as neurotransmitter dopamine abnormalities in attention-deficit/hyperactivity disorder (25).

Nevertheless, a single neurophysiological cause may affect distinct regions. For example, some individuals are prone to abnormal neuronal migration in brain development (ectopias). Such abnormal neuronal migration has been associated with dyslexia (26). However, it is not yet known whether the genetic anomalies that give rise to ectopias in dyslexia may also cause them in other brain regions, thus increasing the risk of other SLDs.

Genetic Basis of SLDs

Most recent genetic work on the etiology of developmental disorders and their co-occurrence

has been conducted using large unselected samples, in which disability is defined and investigated as the low end of ability. Probands (affected individuals) in such studies are selected as extremes of the distribution in any trait of interest in a representative sample. Such research consistently found moderate to high heritability for all cognitive and behavioral traits (27). Research suggests that all cognitive traits are polygenic: influenced by many genes with small effects. On this account, a disorder will be affected by many genes (27). In addition, there is pleiotropy; that is, the same genes may affect multiple traits implicated in diverse cognitive processes, and one gene may depend on the activity of another gene (a process called epistasis).

Twin studies report genetic correlations of 0.2 to 0.7 among SLDs, indicating the extent

to which the same genes are involved in the different conditions. These studies also show that shared environments, such as maternal stressful life events, contribute to comorbidity, especially for autistic traits and attention-deficit behaviors (28).

Nevertheless, a substantial proportion of genetic variance can also be associated with a single domain. For example, in one large twin study of 7-year-olds, some 30% of genetic variance was specific to mathematics (29). However, it remains unknown whether the co-occurrence of reading, math, and other cognitive domains is due to a small set of foundational skills, influenced by both genetics and/or shared environment (see Fig. 2 for an illustration).

Most genes found so far to be associated with cognition seem to work throughout the

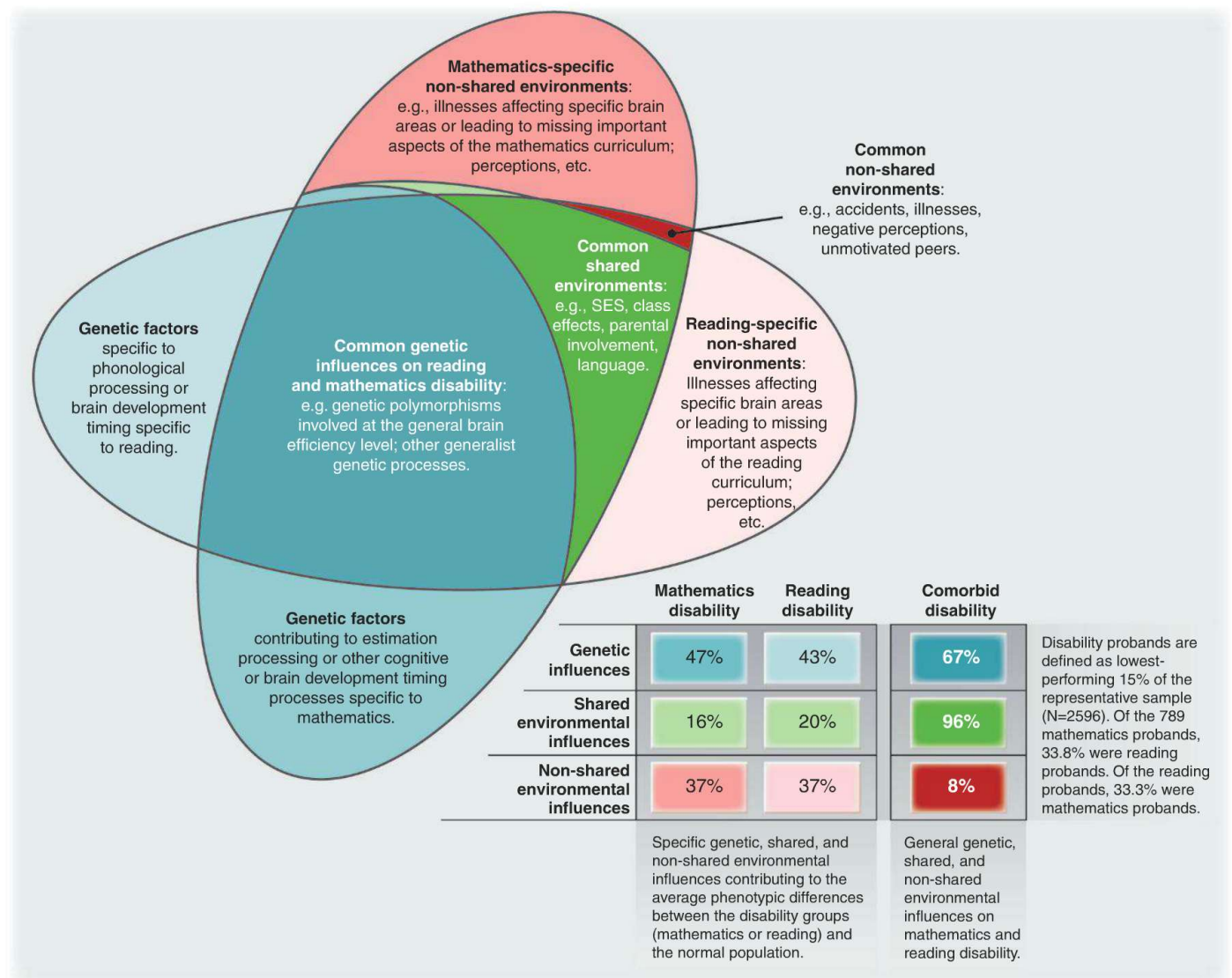


Fig. 2. Etiology of the overlap and specificity of mathematical and reading disabilities. Based on the data from a large-scale twin study (35).

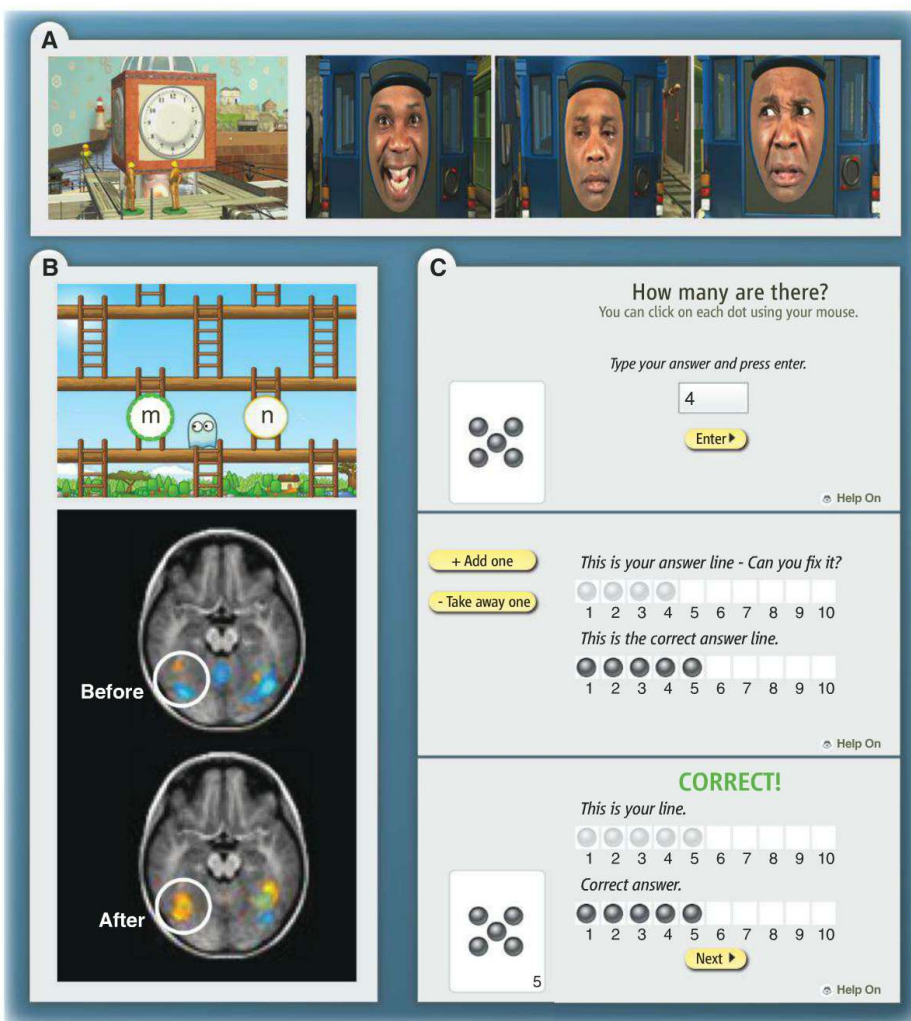


Fig. 3. Examples of adaptive games for SLDs. (A) A still frame from a game to help young autistic spectrum learners to recognize facial emotions. The game uses Transporters: locomotives and cable cars with human faces designed to prevent the learner from avoiding faces (36). (B) Images from Graphogame, a method for teaching early readers and dyslexics letter-sound correspondences. The effects on the brain of 3 hours of training are shown in the brain images (37). (C) An edited sequence of events in the Dots2Track game. (Top) The learner selects a digit corresponding to the number of black dots on the screen. (Middle) Here the answer is incorrect, so the correct number of black dots is counted onto the lower track along with spoken digits, and the response in gray dots is counted down onto the upper line. The learner then has the opportunity to construct the correct answer by adding or taking away a dot until the correct answer is achieved (bottom). The constructive process promotes better learning (38).

Grand Challenges

Develop an understanding of how individual differences in brain development interact with formal education. Investigate how cognitive processes, their neural basis, and their genetic etiology influence the individual's experience of his or her learning environment.

Adapt learning pathways to individual needs. Each child has a unique cognitive and genetic profile. The educational system should be able to monitor and adapt to the learner's current repertoire of skills and knowledge. A promising approach involves the development of technology-enhanced learning applications that are capable of adapting to individual needs for each of the basic disciplines.

distribution, explaining variation in the normal range as well as discriminating probands from the "normal population." Multiple gene variants have been associated with dyslexia (30). However, the associated physiological mechanisms, involving neuronal migration and growth, seem paradoxically general, rather than specific to reading.

Although many genetic abnormalities, such as Down or Williams syndromes affect many aspects of cognition, others can have specific cognitive effects (Fig. 3). Turner's syndrome, for example, is linked with dyscalculia but not with any other SLD (31).

Educational Implications

Although neurodevelopmental disabilities are congenital, they are rarely identified until relatively late in childhood, if at all, because specialized assessments are difficult to access (1) and teachers and parents are often poorly informed about them. Moreover, because of the high rates of co-occurrence, it is likely that an unassessed SLD will be treated as the consequence of the assessed SLD. For example, the dyslexia in a child assessed with attention-deficit/hyperactivity disorder could be assumed to result from that condition and therefore be treated pharmaceutically (e.g., with methylphenidate) but without the specialized help that learners with dyslexia need. Similarly, for a child assessed with dyslexia who also has dyscalculia, a learning program designed to treat the reading disability alone may be implemented. This may be particularly true when one condition is more spectacular or obvious than the other, or indeed when one SLD is more intensively researched than another. For example, National Institutes of Health (NIH) research funding in 2008–2009 for autistic spectrum disorder was 31 times greater than for dyslexia and 540 times greater than for dyscalculia [see the analysis in (32), based on data from <http://projectreporter.nih.gov/reporter.cfm>].

To meet these grand challenges, two important systemic changes will need to take place. First, research into the developmental trajectories of neurocognitive disorders is desperately needed, especially those leading to specific language impairment, dyslexia, and dyscalculia, which are relatively neglected in terms of research funding, despite their impact on the life chances of affected learners (33). But it is also vital to study the even more neglected co-occurrences among SLDs and the educational consequences of co-occurrence. Are the effects of two SLDs additive or multiplicative? What is the etiology of these effects? Better understanding of the etiology will also help with individualizing education for all learners. Second, and informed by the first, teachers, school psychologists, and clinicians need to be trained to identify and understand SLDs and to design learning pathways for each individual sufferer.

References and Notes

- U. Goswami, *Foresight Mental Capital and Wellbeing Project. Learning Difficulties: Future Challenges* (The Government Office for Science, London, 2008).
- H. Tanaka *et al.*, *Psychol. Sci.* **22**, 1442 (2011).
- K. Landerl, A. Bevan, B. Butterworth, *Cognition* **93**, 99 (2004).
- Federal Register, vol. 34 CFR 300.7(c) (10) (U.S. Government, Washington, DC, 1999).
- K. Landerl, K. Moll, *J. Child Psychol. Psychiatry* **51**, 287 (2010).
- C. C. Sexton, H. L. Gelhorn, J. A. Bell, P. M. Classi, *J. Learn. Disabil.* **45**, 538 (2012).
- M. C. Monuteaux, S. V. Faraone, K. Herzig, N. Navsaria, J. Biederman, *J. Learn. Disabil.* **38**, 86 (2005).
- B. St. Pourcain *et al.*, *J. Am. Acad. Child Adolesc. Psychiatry* **50**, 892, e5 (2011).
- C. R. Jones *et al.*, *Neuropsychology* **23**, 718 (2009).
- D. Wechsler, *Wechsler Intelligence Scale for Children. 3rd Edition* (Psychological Corporation, Sidcup, UK, 1992).
- D. Wechsler, *Wechsler Adult Intelligence Scale. 4th Edition* (Psychological Corporation, San Antonio, TX, 2008).
- J. Raven, J. C. Raven, J. H. Court, *Manual for Raven Progressive Matrices and Vocabulary Scales* (Oxford Psychologists Press, Oxford, 1998).
- D. V. M. Bishop, M. J. Snowling, *J. Exp. Child Psychol. Bull.* **130**, 858 (2004).
- J. F. McLean, G. J. Hitch, *J. Exp. Child Psychol.* **74**, 240 (1999).
- J. D. E. Gabrieli, *Science* **325**, 280 (2009).
- E. Paulesu *et al.*, *Science* **291**, 2165 (2001).
- B. Butterworth, S. Varma, D. Laurillard, *Science* **332**, 1049 (2011).
- C. Donlan, in *Why Is Math So Hard for Some Children? The Nature and Origins of Mathematical Learning Difficulties and Disabilities*, D. B. Berch, M. M. M. Mazzocco, Eds. (Paul H. Brookes Publishing, Baltimore, MD, 2007), pp. 151–172.
- American Psychiatric Association, *Diagnostic and Statistical Manual of Mental Disorders* (American Psychiatric Association, Washington, DC, ed. 4, 1994).
- World Health Organization, *International Classification of Diseases 10* (World Health Organization, Geneva, Switzerland, ed. 10, 1994).
- S. Baron-Cohen, A. M. Leslie, U. Frith, *Cognition* **21**, 37 (1985).
- U. Frith, *Q. J. Exp. Psychol. (Hove)* **65**, 2073 (2012).
- D. Gooch, M. Snowling, C. Hulme, *J. Child Psychol. Psychiatry* **52**, 195 (2011).
- K. Landerl, B. Fussenegger, K. Moll, E. Willburger, *J. Exp. Child Psychol.* **103**, 309 (2009).
- J. Swanson, F. X. Castellanos, M. Murias, G. LaHoste, J. Kennedy, *Curr. Opin. Neurobiol.* **8**, 263 (1998).
- A. L. Giraud, F. Ramus, *Curr. Opin. Neurobiol.* **23**, 37 (2013).
- R. Plomin, J. C. DeFries, V. S. Knopik, J. M. Neiderhiser, *Behavioral Genetics* (Worth, New York, ed. 6, 2012).
- K. Dworzynski *et al.*, *Infant Child Dev.* **17**, 121 (2008).
- Y. Kovas, C. Haworth, P. Dale, R. Plomin, *Monogr. Soc. Res. Child Dev.* **72**, 1 (2007).
- T. S. Scerri, G. Schulte-Körne, *Eur. Child Adolesc. Psychiatry* **19**, 179 (2010).
- M. Bruandet, N. Molko, L. Cohen, S. Dehaene, *Neuropsychologia* **42**, 288 (2004).
- D. V. M. Bishop, *PLoS ONE* **5**, e15112 (2010).
- S. Parsons, J. Bynner, *Does Numeracy Matter More?* (National Research and Development Centre for Adult Literacy and Numeracy, Institute of Education, London, 2005).
- J. Morton, U. Frith, in *Manual of Developmental Psychopathology*, D. Cichetti, D. Cohen, Eds. (Wiley, New York, 1995), vol. 1, pp. 357–90.
- Y. Kovas, C. M. A. Haworth, S. A. Petrill, R. Plomin, *J. Learn. Disabil.* **40**, 554 (2007).
- O. Golan *et al.*, *J. Autism Dev. Disord.* **40**, 269 (2010).
- B. D. McCandliss, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 8049 (2010).
- B. Butterworth, D. Laurillard, *ZDM Math. Educ.* **42**, 527 (2010).
- G. Silani *et al.*, *Brain* **128**, 2453 (2005).
- E. B. Isaacs, C. J. Edmonds, A. Lucas, D. G. Gadian, *Brain* **124**, 1701 (2001).
- A. L. Krain, F. X. Castellanos, *Clin. Psychol. Rev.* **26**, 433 (2006).
- E. Proal *et al.*, *Arch. Gen. Psychiatry* **68**, 1122 (2011).
- D. G. Amaral, C. M. Schumann, C. W. Nordahl, *Trends Neurosci.* **31**, 137 (2008).
- T. L. Jernigan, J. R. Hesselink, E. Sowell, P. A. Tallal, *Arch. Neurol. Psychiatry* **48**, 539 (1991).

10.1126/science.1231022

REVIEW

Physical and Virtual Laboratories in Science and Engineering Education

Ton de Jong,^{1*} Marcia C. Linn,² Zacharias C. Zacharia³

The world needs young people who are skillful in and enthusiastic about science and who view science as their future career field. Ensuring that we will have such young people requires initiatives that engage students in interesting and motivating science experiences. Today, students can investigate scientific phenomena using the tools, data collection techniques, models, and theories of science in physical laboratories that support interactions with the material world or in virtual laboratories that take advantage of simulations. Here, we review a selection of the literature to contrast the value of physical and virtual investigations and to offer recommendations for combining the two to strengthen science learning.

Policy-makers worldwide recommend including scientific investigations in courses for students of all ages (1, 2). Research shows advantages for science inquiry learning where students conduct investigations compared

with typical instruction featuring lectures or teacher demonstrations (3, 4). Investigations provide opportunities for students to interact directly with the material world using the tools, data collection techniques, models, and theories of science (1). Physical, hands-on investigations typically fill this need, but computer technologies now offer virtual laboratories where investigations involve simulated material and apparatus. The value of physical laboratories for science learning is generally recognized (1), but the value of virtual, simulated alternatives for hands-on physical laboratories is contested (5). We explore whether

this hesitation concerning virtual laboratories is justified.

Affordances of Physical and Virtual Laboratories

Physical and virtual laboratories can achieve similar objectives, such as exploring the nature of science, developing team work abilities, cultivating interest in science, promoting conceptual understanding, and developing inquiry skills, yet they also have specific affordances (1). Using physical equipment, students can develop practical laboratory skills, including troubleshooting of machinery, and can experience the challenges many scientists face when planning experiments that require careful setup of equipment and observations over long time spans. A related affordance of physical laboratories is that they can take advantage of tactile information that, according to theories of embodied cognition, fosters development of conceptual knowledge [see e.g., (6, 7)].

An important affordance of virtual laboratories is that reality can be adapted. Designers of virtual experiments can simplify learning by highlighting salient information and removing confusing details (8), or they can modify model characteristics, such as the time scale, that make the interpretation of certain phenomena easier (9). Students can conduct experiments about unobservable phenomena, such as chemical reactions, thermodynamics, or electricity (10–13). For example, students can vary the properties

¹Department of Instructional Technology, Faculty of Behavioral Sciences, University of Twente, 7500 AE Enschede, Netherlands.

²Education in Mathematics, Science, and Technology, University of California, Berkeley, Berkeley, CA 94720, USA. ³Department of Educational Sciences, University of Cyprus, Nicosia 1678, Cyprus.

*Corresponding author. E-mail: a.j.m.dejong@utwente.nl

of light rays that travel between a light source and screen (Fig. 1) (14, 15). In virtual laboratories, students can also directly link unobservable processes to symbolic equations and observable phenomena, which encourages them to make abstractions over different representations (16–18).

Virtual experiments offer efficiencies over physical experiments because they typically require less setup time and provide results of lengthy investigations instantaneously (19). This enables students to perform more experiments and thus to gather more information in the same amount of time it would take to do the physical experiment. Physical experiments, however, typically include authentic delays between trials that encourage careful planning and reflection of the next investigation (20).

Finally, in physical investigations students learn about the complexities of science by dealing with unanticipated events, such as measurement errors (21). In contrast, in virtual laboratories students are not distracted by aberrations in the equipment or unanticipated consequences (22). Of course, measurement errors could be modeled in virtual environments, but ensuring that they are authentic would require careful research.

Both physical and virtual investigations succeed when they include worksheets and online and teacher guidance to help students distinguish among their own ideas and the ones demonstrated by the investigation. For example, students benefit when asked to predict the outcome of an experiment and then to compare the result with their own ideas (4, 23, 24). Similarly, students learn how to extract valid information from a complex visualization when they draw what they observed in an experiment about bond breaking (12). For virtual experiments, computer technologies can log student interactions and use the information to diagnose random or uninformative investigations and to prompt students to revise their experimentation strategies and to reflect on their findings (25). Teachers can use logs of student work to flag ideas for class discussion, plan their lessons, identify groups of students who need specialized tutoring, and refine their instruction (4).

Empirical Studies Comparing Physical and Virtual Laboratories

Many well-controlled comparison studies report no differences between physical and virtual laboratories. For example, Wiesner and Lan (26), compared virtual and physical equipment for measuring heat exchange, mass transfer, and humidification and found no differences in the performance of chemical engineering students on a test measuring underlying principles. No differences between virtual and physical experiments on tests of conceptual understanding are

reported by Klahr *et al.* (27) for seventh and eighth graders designing a car or by Zacharia and Constantinou (10) for undergraduates learning about heat and temperature. For measures of inquiry skills, Triona and Klahr (28) found no difference in virtual and physical experiments for fourth and fifth graders investigating the behavior of springs. These studies illustrate that, for acquiring conceptual knowledge, virtual laboratories can replace physical ones. These studies also suggest that tactile information does not appear to be a requirement for the development of conceptual knowledge or inquiry skills with the exception of students, especially young children, who do not have previous relevant physical experience with the phenomenon or concept under study; for example, Zacharia *et al.* (7) found that young children (aged 5 to 6) learning about the working of the balance beam gained more knowledge from physical laboratories than from virtual laboratories.

Many studies show the advantages of virtual, interactive exploration of unobservable phenomena compared with physical experiments of observable phenomena. For example, university students who investigated simulated electric circuits showing moving electrons ac-

quired more conceptual knowledge than those using physical materials (29). Similarly, students using virtual optics materials displaying light rays outperformed those using physical materials (30). Studies show that virtual experiments can enable students to use complex inquiry practices to separate variables that might be difficult to use in physical experiments (17, 27).

The idea that virtual experiments support the acquisition of conceptual knowledge because they produce clean data is also supported in research. For example, first-year secondary students conducting virtual chemistry experiments outperformed those using a physical laboratory on conceptual understanding, which was partly explained by the messy data produced by the physical lab (22).

In the domain of heat and temperature, Zacharia *et al.* (19) found that the use of virtual laboratories offered students more time to experience an experiment and to concentrate on its conceptual aspects than the corresponding physical laboratories, because the virtual laboratories allowed faster manipulation of the materials involved in the experiments of the study's curriculum. On the other hand, this ease of experimentation may also lead to less-structured

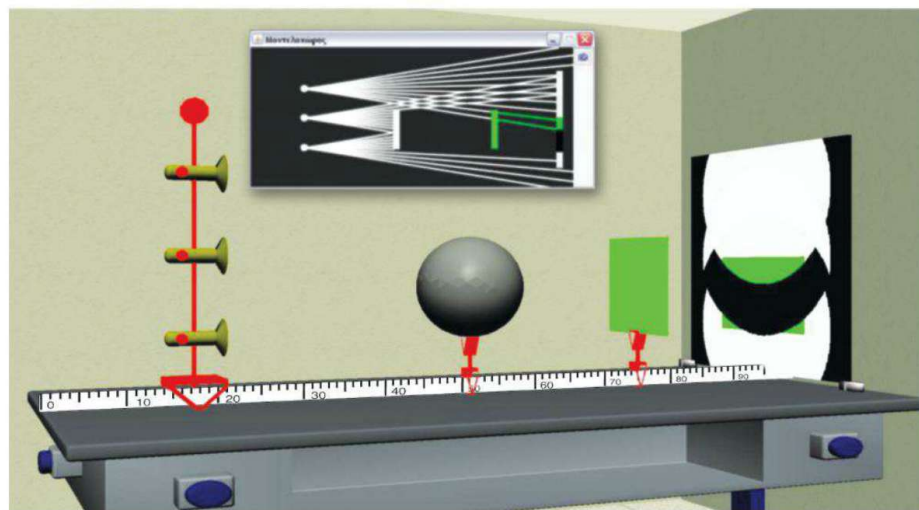


Fig. 1. OptiLab (from the AMAP software) illustrates unobservable light rays to help students understand light's behavior (e.g., how the light rays travel, what happens when light rays reach an obstacle or a colored acetate, and the color of the rays involved). In the OptiLab environment, students are provided with a virtual workbench on which experiments can be performed, virtual objects (e.g., cubes and metal rings) to compose the experimental setup, virtual materials (e.g., colored light sources, different color filters) whose properties are to be investigated, and virtual instruments (e.g., rulers) and displays (e.g., screen). Students could construct their own virtual experimental arrangements by simple and direct manipulation of virtual objects, materials, and instruments. The software allows lighting up to three light sources at a time; adding as many objects as needed between the light source and the screen; changing the distance between objects, materials, and instruments; and changing the angle that the experimental setup could be observed. The software offers feedback throughout the conduct of the experiment by presenting information (e.g., distance and color) through the displays (the screen and the light ray display) of the software.

investigations by students as recently found, in a situation without guidance for experimentation, by Renken and Nunez (20).

These studies show advantages for each type of laboratory, as well as trade-offs. Benefits of virtual laboratories arise when students can investigate unobservable phenomena that are not found in the physical investigation, conduct many more experiments than are possible in the physical setting, link observable and atomic level phenomena, or contrast different depictions of similar phenomena. Physical laboratories have advantages when the instructional goal is to have students acquire a sophisticated epistemology of science, including the ability to make sense of imperfect measurements and to acquire practical skills.

Combining Physical and Virtual Laboratories

Combinations of physical and virtual experiments can capitalize on the features of each approach. Huppert *et al.* (31), for example, found that a group of microbiology students who car-

ried out physical laboratories were less successful on a conceptual test than a group where a simulation was substituted for one laboratory session. Zacharia *et al.* (19) found that students conducting a physical laboratory and a virtual laboratory outperformed students doing the physical laboratory on conceptual understanding of heat and temperature. Kolloff and de Jong (16) found that vocational engineering students who did a combination of virtual and physical laboratory were more successful than those doing a physical laboratory alone on both conceptual and procedural knowledge of electric circuits. Climent-Bellido, Martínez-Jiménez *et al.* (32) compared chemistry students who used a physical laboratory with students who used a simulation of distillation preceding the physical laboratory and found an advantage for the combination. Olympiou and Zacharia (30) studied freshmen students learning about optics under three conditions: only virtual, only physical, and a combination. Students in the combined condition outperformed those in the physical alone

and virtual alone conditions, attesting the value of the combination over both other conditions.

Also, Jaakkola *et al.* (11) found an advantage for students who conducted physical and virtual experiments over those conducting virtual experiments alone for sixth grade students learning about electric circuits on a measure of conceptual knowledge. Jaakkola *et al.* (33) analyzed videotapes of students as they were learning and found that, in the combination condition, students profited from comparing two potentially different representations of the same phenomena and using abstract reasoning to analyze the differences.

Combinations of virtual and physical experiments have succeeded independently of the order of investigation. Toth *et al.* (21) studied DNA gel electrophoresis and found a small but nonsignificant advantage for starting with the virtual laboratory and then follow a physical laboratory compared with following the reverse order. Chini *et al.* (34) studied conceptual understanding of pulleys and found no differences

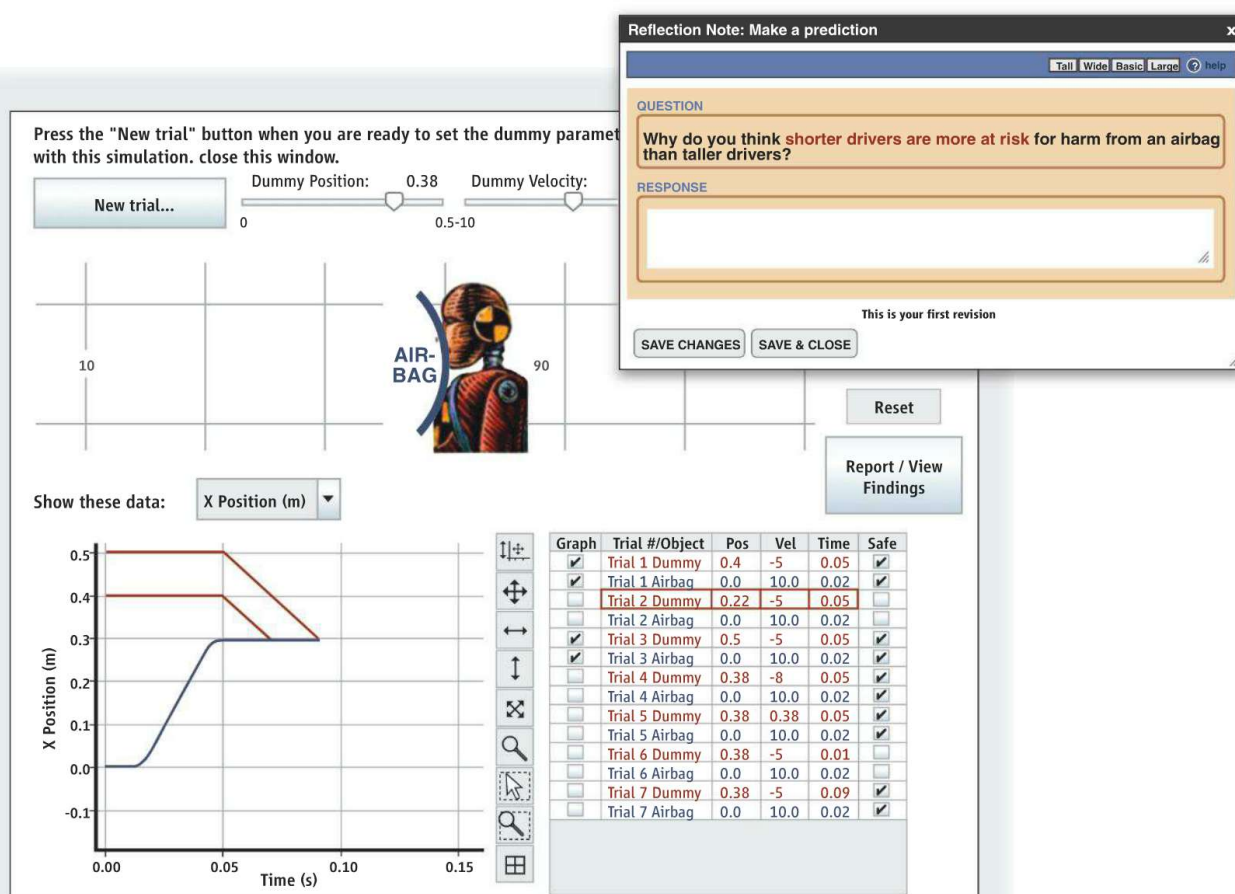


Fig. 2. Air bags unit. In the air bags Web-based Inquiry Science Environment (WISE) unit, students conduct virtual experiments to explore a professional laboratory: conditions leading to injuries from an air bag deployed in a car crash. Students make predictions about the variables (such as the

driver's height), test their conjectures, interpret graphs of the results, review the record of their experiments, and explain their reasoning. Teachers use online tools to monitor progress of their students, flag student work for discussion, and give online guidance.

Grand Challenges

Create online environments that use stored data from individual students to guide them to virtual experiments appropriate for their stage of understanding. In this way, online environments can provide students with personalized guidance to maximize outcomes.

Determine the ideal balance between virtual and physical investigations for courses in different subject areas. Although the best combination may vary based on circumstances, combining both virtual and physical investigation is likely to be optimal.

Identify the skills and strategies teachers need to implement a science curriculum featuring virtual and physical laboratories. The aim is to create a professional development program that enables teachers to revise their lessons based on information obtained from student online work.

between a virtual-physical and a physical-virtual sequence. Overall, well-designed combinations of virtual and physical experiments compared with either one alone allow students to gain a more nuanced understanding of scientific phenomena and a more robust understanding of inquiry.

Conclusions

Both virtual and physical investigations can meet the goals for investigation in science courses. Both approaches allow students to use tools, data-collection techniques, models, and theories of science (1). Virtual experiments delivered with computer technology add value to physical experiments by allowing students to explore unobservable phenomena; link observable and unobservable phenomena; point out salient information; enable learners to conduct multiple experiments in a short amount of time; and provide online, adaptive guidance. Combinations of virtual and physical laboratories offer advantages that neither one can fully achieve by itself.

In addition to their virtues for promoting conceptual knowledge, virtual laboratories have additional advantages such as offering cost-effective alternatives to physical laboratories for topics such as DNA gel electrophoresis (21). They also give students the opportunity to use experimental systems that are beyond the reach of schools [as is illustrated for the air bags project (Fig. 2)] (17). In addition, they can enable students to investigate conjectures that are not possible in physical experiments by, for example, changing the magnetic field of the earth, varying accumulation of greenhouse gases, or studying the impact of extreme heart rate and blood pressure.

Virtual investigations can equal or exceed the impact of physical investigations on measures of conceptual understanding but the excitement of conducting hands-on experiments also deserves attention. Studies comparing virtual and physical experiments have primarily measured impacts on conceptual understanding of scientific phenomena and inquiry practices, but other outcomes, such as interest in science as a career, are worthy of investigation.

Research on virtual and physical laboratories calls for nuanced decision-making (27). Clearly, there are times when virtual investigations could be equal to or more effective than physical investigations and times when physical laboratories are most appropriate. Designers of instruction can improve outcomes by taking advantage of the affordances of each type of laboratory. However, design of guidance to ensure that students benefit from laboratories remains the most crucial variable in the success of science instruction (35). To design laboratories that take advantage of powerful guidance requires interdisciplinary teams involving domain experts, technologists, and learning scientists. Such teams typically refine their designs based on trials in instructional settings.

More opportunities to take advantage of virtual or online investigations arise regularly and deserve further study. For example, funding from the European Union is making data from facilities such as the CERN particle accelerator or the European Space Agency's satellites available for use in schools (36). In addition, new technologies are increasing access to remote laboratories [e.g., (36, 37)], raising new questions for researchers.

References and Notes

1. National Research Council, *America's Lab Report: Investigations in High School Science*, S. R. Singer, M. L. Hilton, H. A. Schweingruber, Eds. (National Academy Press, Washington, DC, 2006).
2. M. Rocard et al., *Science Education Now: A Renewed Pedagogy for the Future of Europe* (European Commission: Directorate-General for Research, Brussels, 2007).
3. D. D. Minner, A. J. Levy, J. Century, *J. Res. Sci. Teach.* **47**, 474 (2010).
4. M. C. Linn, H.-S. Lee, R. Tinker, F. Husic, J. L. Chiu, *Science* **313**, 1049 (2006).
5. National Science Teachers Association, The integral role of laboratory investigations in science instruction (2007); www.nsta.org/about/positions/laboratory.aspx.
6. L. W. Barsalou, *Annu. Rev. Psychol.* **59**, 617 (2008).
7. Z. C. Zacharia, E. Loizou, M. Papaevripidou, *Early Child. Res. Q.* **27**, 447 (2012).
8. K. C. Trundle, R. L. Bell, *Comput. Educ.* **54**, 1078 (2010).
9. D. N. Ford, D. E. M. McCormack, *Simul. Gaming* **31**, 309 (2000).

10. Z. C. Zacharia, C. P. Constantinou, *Am. J. Phys.* **76**, 425 (2008).
11. T. Jaakkola, S. Nurmi, K. Veermans, *J. Res. Sci. Teach.* **48**, 71 (2011).
12. Z. H. Zhang, M. C. Linn, *J. Res. Sci. Teach.* **48**, 1177 (2011).
13. L. Deslauriers, C. E. Wieman, *Phys. Rev. Spec. Topics Phys. Educ. Res.* **7**, 010101 (2011).
14. G. Olympiou, Z. Zacharias, T. de Jong, *Instr. Sci.* **41**, 575 (2013).
15. E. Hatzikraniotis, G. Bisdikian, A. Barbas, D. Psillos, in *8th International Conference on Computer Based Learning in Science (CBLIS)*, C. P. Constantinou, Z. C. Zacharia, M. Papaevripidou, Eds. (Heraklion, Crete, Greece, 2007), pp. 523–530.
16. B. Kolloffel, T. de Jong, *J. Eng. Educ.*; <http://users.edte.utwente.nl/jong/kolloffeldejongJEE.pdf>.
17. K. W. McElhaney, M. C. Linn, *J. Res. Sci. Teach.* **48**, 745 (2011).
18. J. van der Meij, T. de Jong, *Learn. Instr.* **16**, 199 (2006).
19. Z. C. Zacharia, G. Olympiou, M. Papaevripidou, *J. Res. Sci. Teach.* **45**, 1021 (2008).
20. M. D. Renken, N. Nunez, *Learn. Instr.* **23**, 10 (2013).
21. E. E. Toth, B. L. Morrow, L. R. Ludvico, *Innovative High. Educ.* **33**, 333 (2009).
22. K. Pyatt, R. Sims, *J. Sci. Educ. Technol.* **21**, 133 (2012).
23. T. de Jong, *Science* **312**, 532 (2006).
24. M. Windschitl, T. Andre, *J. Res. Sci. Teach.* **35**, 145 (1998).
25. T. de Jong et al., *Br. J. Educ. Technol.* **41**, 909 (2010).
26. T. F. Wiesner, W. Lan, *J. Eng. Educ.* **93**, 195 (2004).
27. D. Klahr, L. M. Triona, C. Williams, *J. Sci. Teach.* **44**, 183 (2007).
28. L. M. Triona, D. Klahr, *Cogn. Instr.* **21**, 149 (2003).
29. N. D. Finkelstein et al., *Phys. Rev. Spec. Topics Phys. Educ. Res.* **1**, 010103 (2005).
30. G. Olympiou, Z. C. Zacharia, *Sci. Educ.* **96**, 21 (2012).
31. J. Huppert, S. M. Lomask, R. Lazarowitz, *Int. J. Sci. Educ.* **24**, 803 (2002).
32. M. S. Climent-Bellido, P. Martínez-Jiménez, A. Pones-Pedrajas, J. Polo, *J. Chem. Educ.* **80**, 346 (2003).
33. T. Jaakkola, S. Nurmi, E. Lehtinen, in *Use of External Representations in Reasoning and Problem Solving*, L. Verschaffel, E. de Corte, T. de Jong, J. Elen, Eds. (Routledge, New York, 2010), pp. 133–153.
34. J. J. Chini, A. Madsen, E. Gire, N. S. Rebello, S. Puntambekar, *Phys. Rev. Spec. Topics Phys. Educ. Res.* **8**, 010113 (2012).
35. L. Alfieri, P. J. Brooks, N. J. Aldrich, H. R. Tenenbaum, *J. Educ. Psychol.* **103**, 1 (2011).
36. D. Gillet, T. de Jong, S. Sotiriou, C. Salzmann, paper presented at the IEEE EDUCON, Berlin, 13 to 15 March 2013.
37. A. Mejías Borrero, J. Andújar Márquez, *J. Sci. Educ. Technol.* **21**, 540 (2012).

Acknowledgments: The contribution by T.d.J. and Z.C.Z. was partially funded by the European Union in the context of the Go-Lab project (grant agreement no. 317601) under the Information and Communication Technologies (ICT) theme of the 7th Framework Programme for R&D (FP7). This document does not represent the opinion of the European Union, and the European Union is not responsible for any use that might be made of its content. M.C.L.'s contribution is based on work supported, in part, by the NSF under grants DRL-1119670: Continuous Learning and Automated Scoring in Science (CLASS); DRL-0918743: Visualizing to Integrate Science Understanding for All Learners (VISUAL); and DRL-0733299: Logging Opportunities in Online Programs for Science (LOOPS). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the NSF.

10.1126/science.1230579

PERSPECTIVE

Teacherpreneurs: A Bold Brand of Teacher Leadership for 21st-Century Teaching and Learning

Barnett Berry

Challenges facing our public schools demand a bold brand of teacher leadership. Teacherpreneurs, effective teachers who teach students regularly but also incubate and execute the kinds of policies and pedagogies students deserve, represent a new culture of training and ingenuity. Teachers who lead outside the classroom but do not lose their connection to students are best positioned to develop and disseminate best policies and practices for 21st-century teaching and learning.

Since the release of the Coleman Report in 1966, there has been a steady drip of empirical evidence showing that teachers are the most crucial in-school factor for student learning (1–4). And while U.S. policy-makers have sought to reform education by improving teacher quality, in doing so they have not paid a great deal of attention to the research on teaching and learning. Making matters more complicated, America's approach to the teaching profession has been driven more by ideological agendas and power politics than by scientific evidence.

Just as dusty blackboards still hang in some classrooms, many reformers fail to envision schools that look different than they did when they were students. We have entered a new era with advanced learning technologies (5) and growing scientific evidence on how humans learn, with enormous implications for teachers and teaching (6). New methods of assessing cognition, emotion, and learning make it possible for teachers, if they are well prepared and supported, to serve students in ways previously unimaginable (7, 8).

Although teachers are paramount to student learning, too few education policies promote leadership from those who teach.

Why We Need a Bold New Brand of Teacher Leadership

Isolated behind the closed doors of individual classrooms, teachers traditionally have had little time to observe and learn from their peers. However, education research demonstrates that when teachers collaborate with one another, their students' achievement improves (9). Economists, using sophisticated statistical methods and large databases, have concluded that students score higher on achievement tests when their teachers have opportunities to work with colleagues over long periods of time and spread their expertise (10). And in the 2009 MetLife Survey of the American

Teacher, over 90% reported that their colleagues contribute to their individual teaching effectiveness (11).

Richard Elmore made the compelling case that many education policies and practices often wither, primarily because reformers fail to “develop organizational structures that intensify and focus” the new reforms supported by too few “intentional processes for [the] reproduction of successes (12).” His research pointed out that reform is about learning, and for teachers to teach more effectively they must have “encouragement and support, access to special knowledge, time to focus on the requirements of the new task, time to observe others doing it (12).”

But these conditions rarely are in place. Compared to teachers in nations with top-performing education systems, like Singapore, most U.S. teachers have very limited access to leadership and learning opportunities or time to engage in high-quality professional development—such as the “lesson study” common in Singapore and Shanghai (13). Because of the hierarchical structure of U.S. schools, teachers who want to lead outside their



Teacherpreneurs lead in many capacities inside and outside the classroom. Though they are exceptional teachers, they are not the exception.

Center for Teaching Quality, 605 West Main Street, Carrboro, NC 27510, USA.

E-mail: bberry@teachingquality.org

Photos courtesy of Barbara Kinney, Renee Moore, Ariel Sacks, and Alan Wrieder

own classroom have had to leave it to become administrators, district leaders, or policy-makers.

The Coming Age of Teacherpreneurs

America's public education system needs teacherpreneurs—classroom experts who teach students regularly, but also have time, space, and reward to spread their ideas and practices to colleagues as well as administrators, policy-makers, parents, and community leaders. The Center for Teaching Quality has supported as well as documented how this special brand of teacher leaders has begun to serve as online coaches, edugame developers, community organizers, and policy analysts, without leaving the classroom (14). In doing so, they have begun to solve problems of student and teacher learning that today's reformers have yet to identify. Daunting barriers remain, including the relatively large number of educators in school systems who never teach, the highly prescriptive teaching day, and top-down reformers whose political agendas are out of sync with the ideas of classroom experts. However, teacherpreneurs, because of their deep knowledge of students, families, and communities, are more likely to be embraced by their colleagues.

I am optimistic. Most Americans have trust and confidence in individual teachers (15), and new technologies that amplify teachers' collective wisdom and the impact of their leadership will resonate with parents and the public. Addi-

tionally, MetLife's most recent survey revealed that one in four teachers nationwide are extremely or very interested in hybrid roles that would allow them to both teach and lead outside their schools, districts, and states (16).

While these classroom experts should be highly paid, teacherpreneurship is not mainly about establishing a new income stream for underpaid professionals. It is much more about rewarding a new culture of schooling and creativity. As Peter Drucker said of entrepreneurs almost 50 years ago, "search for change, respond to it and exploit opportunities (17)." It is time for America to cultivate teacherpreneurs who will do the same, deepening and spreading best policies and practices for 21st-century teaching and learning.

References

1. J. S. Coleman *et al.*, *Equality of Educational Opportunity* (U.S. Office of Education, U.S. Government Printing Office, Washington, DC, 1966).
2. R. Strauss, E. Sawyer, *Econ. Educ. Rev.* **5**, 41 (1986).
3. R. F. Ferguson, *Harvard J. Legis.* **28**, 465 (1991).
4. C. T. Clotfelter, H. T. Ladd, J. Vigdor, "Teacher credentials and student achievement in high school: A Cross subject analysis with student fixed effects," Working paper series, no. 13617 (National Bureau of Economic Research, Stanford, 2007).
5. S. Cavaenagh, *Educ. Week* **32**, 7 (2013); www.edweek.org/ew/articles/2013/01/03/15cyber.h32.html.
6. L. Darling-Hammond, J. Bransford, *Preparing Teachers for a Changing World: What Teachers Should Land Be Able to Do* (Jossey-Bass, San Francisco, 2005).

7. National Research Council, *How People Learn: Brain, Mind, Experience, and School: Expanded Edition* (National Academies Press, Washington, DC, 2000).
8. J. E. Zell, *The Art of Changing the Brain: Enriching the Practice of Teaching by Exploring the Biology of Learning* (Stylus, Sterling, VA, 2002).
9. S. J. Rosenholtz, *Teachers' Workplace: The Social Organization of Schools* (Teachers College Press, New York, 1991).
10. C. K. Jackson, E. Bruegmann, *Teaching Students and Teaching Each Other: The Importance of Peer Learning for Teachers* (National Bureau of Economic Research, Stanford, 2009); www.nber.org/papers/w15202.
11. D. Markow, A. Pieters, *The MetLife Survey of the American Teacher: Collaborating for Student Success* (MetLife Foundation, New York, 2010).
12. R. F. Elmore, *Harv. Educ. Rev.* **66** (Spring 1996); <http://ed-share.educ.msu.edu/scan/TE/danagnos/te9206B.PDF>.
13. OECD, *Lessons from PISA for the United States: Strong performers and successful reformers in education*; www.oecd.org/pisa/46623978.pdf.
14. B. Berry, A. Byrd, A. Wieder, *TEACHERPRENEURS: Innovative Teachers Who Lead But Don't Leave* (Jossey-Bass, San Francisco, 2013).
15. B. Bushaw, S. Lopez, Public education in the United States: A nation divided. *Kappan* (2012); www.pdkintl.org/poll/docs/2012-Gallup-poll-full-report.pdf.
16. MetLife Corporation, *The MetLife Survey of the American Teacher: Challenges for School Leadership* (2013); <https://www.metlife.com/assets/cao/foundation/MetLife-Teacher-Survey-2012.pdf>.
17. R. Branson, What is your definition of entrepreneur? (Web log post, 30 October 2012); www.linkedin.com/today/post/article/20121030140136-204068115-what-is-your-definition-of-entrepreneur?trk=eml-mktg-condig-118-p1.

10.1126/science.1230580

REVIEW

Professional Development for Science Teachers

Suzanne M. Wilson

The Next Generation Science Standards will require large-scale professional development (PD) for all science teachers. Existing research on effective teacher PD suggests factors that are associated with substantial changes in teacher knowledge and practice, as well as students' science achievement. But the complexity of the U.S. educational system continues to thwart the search for a straightforward answer to the question of how to support teachers. Interventions that take a systemic approach to reform hold promise for improving PD effectiveness.

Calls for improving science education in the United States, such as raising standards for all children and focusing the curriculum, are loud and clear. The Next Generation Science Standards (NGSS) [www.nextgenscience.org (1)] press for a vision of science teaching that emphasizes students' active engagement in genuine scientific problems, a commitment to "less is more," and an approach to make science appealing to all students. Of central importance are scientific practices and the integration of students'

learning of core disciplinary concepts with active engagement in doing science (2). In addition, an increased emphasis on studying engineering is integrated throughout this new vision of science teaching and learning. Helping current teachers acquire the knowledge, skill, and will to meet these new standards is a daunting enterprise requiring large-scale professional development (PD) of high quality that is adaptable across myriad contexts.

Teachers in the United States have access to a wealth of PD opportunities, including summer institutes, coaching, mentoring, school-based professional learning communities, research experiences with practicing scientists, and "make-and-take" events that introduce teachers to new materials.

Teachers study together, conduct inquiries, read research, learn new technologies, navigate multimedia environments, and read cases. These PD programs have different goals: Traditionally, much PD has focused on enriching teachers' content knowledge (CK), introducing new curriculum and instructional materials, enhancing pedagogical CK, or educating them about scientific inquiry. The U.S. PD system is a carnival of options.

Research on Science PD

Carefully designed research, drawing from a range of disciplinary approaches from ethnographies to randomized clinical trials, has begun to shed light on what makes for effective PD. Five general characteristics have been identified: (i) focusing on specific content, (ii) engaging teachers in active learning, and (iii) enabling the collective participation of teachers (sometimes administrators), as well as (iv) coherence (aligned with other school policy and practice) and (v) sufficient duration (both in intensity and contact hours) (3–6).

Researchers have nominated five additional factors for effective PD: (i) activities are close to practice (7), (ii) participants' physical and psychological comfort is taken into account (8), (iii) teachers are immersed in inquiry experiences and witness models of inquiry teaching (6), (iv) curriculum materials are educative for teachers and students (9, 10), and (v) teachers receive direct instruction in the teaching specified in inno-

Department of Teacher Education, Michigan State University, East Lansing, MI 48824, USA.

E-mail: swilson@msu.edu

vative materials (11). Repeatedly, the importance of strong principal support is emphasized (12).

Recently, there has been an effort to test these “best practices” with rigorous research. Here, I consider meticulously peer-reviewed literature. The U.S. Department of Education funded two clinical trials to examine the effects of PD in mathematics and literacy. One of these trials examined the impact of two research-based PD interventions for reading: (i) a content-focused teacher institute series that began in the summer and continued over the school year and (ii) the same institute series plus in-school coaching (13). The sample included 90 schools in six districts (270 teachers), with equal numbers of schools in each district randomly assigned to one of the two treatments or to the control group, which participated only in the usual district PD. Teachers in treatment schools scored significantly higher on the teacher knowledge test than did teachers in control schools; teachers in the treatment schools also used explicit instruction more often. However, neither PD intervention resulted in significantly higher student test scores, and there was no additional benefit of the PD enhanced by the coaching. One year later, there were no major lasting effects on teacher or student outcomes. Similar results were found in a study of PD for mathematics teachers (14).

A handful of experimental studies have focused on science-specific PD. Using a randomized experimental design in 39 school districts in six states, researchers compared the effects of three systematically varied teacher interventions that are common forms of PD—teaching cases, looking at student work, and metacognitive analysis (as well as “business as usual” as a control condition)—on teacher and student science CK (15). Participating districts had to be well established and stable, with strong science leadership; 270 elementary teachers and 7000 students participated in the study. Each intervention involved a 14-week electric circuits course offered in 3-hour sessions. Hierarchical linear models were fitted to gain scores from pre- to posttest for students and from pretest to follow-up test for teachers. Separate models analyzed teacher and student outcomes. Researchers found that each PD course significantly increased teacher and student science test scores, and the effects held one year later. However, only PD that involved teachers examining student thinking and considering the implications for instruction was associated with increases in both teacher and student science knowledge.

In the Science Teachers Learning through Lesson Analysis (STeLLA) project, teachers participated in PD that involved analyzing science teaching practice using video cases (16). Two groups of elementary teachers participated in a 3-week PD summer institute (SI) designed to enhance their CK. The STeLLA group ($n = 32$ participants) also analyzed video cases during the SI and throughout the school year. Sixteen teachers participated in the content-focused SI only. The program sub-

stantially improved teachers’ CK and their ability to analyze science teaching. Students of STeLLA teachers also demonstrated considerably higher gains in their science CK. Teachers who participated in the content-only SI demonstrated initial gains in CK immediately after the summer but were unable to maintain those gains through the school year.

In another study, researchers conducted an efficacy study of three PD programs in a large urban district: investigating Earth sciences, Earth sciences by design, and a hybrid of the two (11). Teachers experienced one of four conditions that differed along two dimensions: (i) whether teachers experienced PD that provided guidance in selecting relevant curricular materials and included inquiry-oriented instruction and (ii) whether teachers received PD that included direct instruction in teaching practices. Researchers found that PD with direct instruction in teaching practices can increase students’ knowledge of Earth science, but there were mixed results concerning the claim that helping teachers select curriculum that is coherently aligned with standards and includes inquiry-oriented activities will lead to increased student learning.

Although there are a growing number of research studies that examine the effectiveness of the five features of PD described above, the evidence is uneven, and we lack clear direction. Why the uneven empirical base? If no curriculum is teacher-proof, it seems obvious that no curriculum is context-proof. Resources, students, leadership, and teacher knowledge shape an intervention’s implementation. Even when interventions are implemented with high fidelity, schools are subject to forces beyond researchers’ control. District leadership churns, new curriculum and assessments arrive, policies change, and resources are cut. These factors vex researchers who need to control these changes as much as possible.

Another reason for the uneven research base is that we lack sound measures of teachers’ knowledge of science for the classroom, as well as aligned measures of students’ understanding represented in the NGSS. Currently available measures of student learning focus largely on the mastery of scientific facts but do not assess students’ conceptual understanding or their abilities to engage in scientific practices, which are the foci of much PD.

A third issue is that much PD does not identify specific instructional practices that teachers can master, nor are teachers given the time and structures needed for repeated practice and sufficient reflection (17). Instead, teachers learn about general approaches to instruction and take those ideas back to their classrooms, where they may (or may not) try to implement some of these methods. In one study, researchers found that two PD programs focusing on scientific inquiry led to changes in teachers’ practice, but the experimental impacts corresponded to the features of scientific inquiry that the teachers were exposed to the

most (18). Both PD programs emphasized questioning and gathering evidence, but neither effort uniformly engaged teachers in other clearly specified features of inquiry, such as formulating and justifying explanations and connecting those explanations to scientific knowledge. Most troubling was the finding that students in one program had lower standardized test scores than students in a control group in the first year of the study. Researchers hypothesized that learning to ask scientific questions and gather evidence without connecting those activities back to scientific explanations and concepts might leave students and teachers more confused than before. An alternative explanation might be that PD that fails to clearly explicate different aspects of an instructional regime in detail—and to provide sufficient opportunity for repeated practice with focused feedback—will be less effective.

Additionally, researchers lack a clear theory of the underlying mechanisms involved in teacher learning. For instance, researchers have argued that teachers’ increased CK leads to better self-efficacy (19). In turn, this increased efficacy leads to higher levels of persistence. Thus, teachers who increase their CK also improve their confidence, which leads to more motivation and perseverance as teachers learn to educate in fundamentally different ways. The five general characteristics listed above—duration, active learning, collective participation, coherence, and content focus—are design features, but future research will need to explore how these features work together to produce teacher learning. Using the above example, does a focus on content lead to greater teacher confidence, which, in turn, leads to greater active engagement and, eventually, higher student achievement?

A more complex view of teacher learning is clearly needed, one in which professional learning is seen as more dynamic and iterative, connecting teachers’ experiences in their classrooms with formal opportunities for collective reflection and for acquiring new knowledge that targets genuine problems of practice (17). Models of teacher learning should also account for the internal coherence of a school’s leadership, culture, curriculum, assessments, and PD, as teachers learn inside of organizations that fundamentally shape their interests in and abilities to learn from practice (20).

Although researchers have identified several features of effective PD, rigorous research has yet to produce conclusive support for those characteristics. Problems include a lack of sound measures and a strong theoretical understanding of the mechanisms of teacher learning.

PD Embedded in School Reform

An understanding of how PD fits into the educational system’s larger ecology is also needed. PD may be best understood as nested within an environment that includes schools (which have

their own cultures and norms), districts (which have their own leadership and capacity), students and their families (who have their own backgrounds and expectations), teachers (who have their own knowledge and experience), and curricular materials and other resources. This view has led reformers to create education reform efforts that take a systems approach to improvement, considering leadership, curricula and assessments, PD, organizational infrastructure, and human and social capital. In a longitudinal study of school change, one research team identified five supports for change: (i) leadership (principals who are strategic, focused on instruction, and inclusive), (ii) professional capacity (teacher quality, their beliefs about change, their capacity to work collaboratively, and the quality of ongoing professional development), (iii) parent-community ties (schools that are welcoming to parents and have strong connections to local institutions), (iv) student-centered learning environments (schools that are safe, nurturing, stimulating, and welcoming), and (v) instructional guidance (the organization of the curriculum, its academic rigor, and the tools teachers have to advance learning) (21). The researchers found that the real value of these supports was in their combined strength: Schools with strength in three to five of these supports were 10 times more likely to demonstrate significant learning gains (as measured in mathematics and reading).

Initiatives that take a systemic view of educational improvement present challenges to those who want to draw causal conclusions about the contributions of specific components of the reform to student learning. Teachers' knowledge and perceptions, administrative support, PD, and available resources, among other factors, are intertwined with student experiences with inquiry, teachers' collaborations, technology use, and other aspects of the system. It is nearly impossible to isolate the effects of PD on student learning.

Pressing Challenges

Going to scale with effective PD for the entire teacher workforce of 3.7 million will require more research. We face several looming challenges: preparing educators to teach the NGSS, meeting the needs of English-language learners (ELLs), and harnessing new communication technologies to produce quality online science-specific PD that is both effective and widely available.

The NGSS present a view of science teaching that differs from the standard fare in U.S. classrooms. Getting from here to there will require considerable investment of resources: The research described here required considerable development of highly specified instructional materials and tools to support teachers and students in using those materials; additionally, this research validated ongoing assessments of learning and responsive, extensive PD. Responding to the NGSS will require the development of many more such resources, and

this innovation will need a coordinated system of research to empirically document the hallmarks of effective instruction, the qualities of effective materials, and the dynamics of high-quality PD. Although the array of available PD in this country is extraordinary, we cannot afford such broad experimentation without learning from it so that we can much better align the resources spent on PD (estimates range from \$1 billion to \$4 billion per year) with the demands teachers face in today's classrooms.

We especially cannot afford the high cost of experimentation, given the changing U.S. demographics. At least 21% of our students are ELLs, and helping them engage in science is a pressing problem for all K-12 science teachers. Only 2% of eighth-grade ELLs achieved at or above proficiency levels on the 2009 National Assessment of Educational Progress in science (compared with 32% of English-speaking students) (22). Only 12% of practicing teachers reported that they had any training in teaching ELLs. The percentages are probably much lower for teachers who have had PD in teaching science to ELLs.

Recent research has begun to explore this challenge by offering PD that integrates learning science and literacy. One such intervention focused on promoting the learning of science inquiry by students from linguistically and culturally diverse backgrounds (23). The study investigated the impact of an inquiry-based intervention on students' ability to conduct inquiry. The intervention involved units for grade 3 (measurement and matter) and grade 4 (water cycle and weather). Across the year, teachers attended four full-day workshops that covered scientific inquiry, instructional scaffolding, and balancing teacher guidance and student initiative. Teachers worked on unit plans, demonstrated lessons, and shared ideas. Other workshop topics included incorporating students' home languages and cultures into instruction, as well as

incorporating language and literacy into science. Seven teachers in six elementary schools in a large urban district participated in a field study that investigated the PD's effects on students' understanding of inquiry. Researchers documented a significant growth in students' understanding of inquiry from pre- to posttest. In another project, researchers found that when teachers participated in a 3-year effort to provide both curriculum materials and PD designed to improve teaching practices, the science achievement scores of third- to fifth-graders were significantly raised, and achievement gaps among demographic subgroups decreased consistently for third- and fifth-graders and held steady for fourth-graders (24). Other researchers report on a quasi-experimental study of a science intervention that targeted ELLs and low-socioeconomic status non-ELLs (25). Students who participated in the treatment classrooms demonstrated significantly higher achievement on district benchmark tests in science and reading, as well as on the state reading exam, but not on state science tests.

Another challenge concerns the accessibility of high-quality PD. In a recent survey, the majority of science teachers reported that they were provided more opportunities for generic PD than science-specific PD (24). In this case, new technologies might hold promise. Online PD has the potential for providing "just-in-time assistance" and is potentially more scalable than PD that presses on limited local resources. In addition to online courses, other emerging environments—such as multi-user virtual environments in which the participants take on avatars in virtual worlds, augmented realities in which participants in their own real-world contexts interact with a virtual setting, and social networks that connect teachers across the country—also hold promise for increasing teachers' access to relevant, high-quality science PD and materials (26). To date, not enough research exists

Grand Challenges

Identify the underlying mechanisms that make some teacher professional development (PD) programs more effective than others. Rigorous research on effective PD for science teachers is gradually accumulating, but we need a stronger theoretical base that reflects the complex ecology in which teachers work and learn. We also need better measures and interventions that are more highly specified to meet particular teacher needs.

Identify the kind of PD that will best prepare teachers to meet the challenges of the Next Generation Science Standards (NGSS). The NGSS specify an entirely new way of teaching science in the United States. It will require a considerable investment of resources to develop appropriate instructional materials and the tools needed to support teachers and students in using those materials. We must realign the considerable resources spent on PD with the demands teachers will face in a NGSS classroom, and we cannot afford such broad experimentation without funding the research required to learn from it.

Harness new technologies and social media to make high-quality science PD available to all teachers. Online PD has the potential for providing "just-in-time assistance," and it is potentially more scalable than PD that relies on limited local resources. To date, there exists little research to help us understand the affordances and limits of these venues.

to help us understand the affordances and limits of these venues.

In sum, rigorous research on effective PD for science teachers is gradually accumulating, but we need a stronger theoretical base that reflects the complex ecology in which teachers work and learn. We also need better measures and interventions that are more highly specified. Though some might hope for a silver bullet, education reform that leads to fundamental change, such as that envisioned in the NGSS, requires time [it takes several years for teachers to change their practice (6, 27, 28)]. Reform efforts also require investments in infrastructure (leadership, teacher networks, planning time), the organizational coherence that encourages teachers to take risks and learn new content, parents to support the new standards, and students to demonstrate the perseverance and curiosity needed to achieve scientific literacy.

References

1. National Research Council (NRC), *A Framework for K-12 Science Education: Practices, Crosscutting Concepts, and Core Ideas* (Board on Science Education, NRC, Washington, DC, 2012).
2. NRC, *Taking Science to Schools* (Board on Science Education, NRC, Washington, DC, 2007).
3. L. M. Desimone, *Educ. Res.* **38**, 181 (2009).
4. L. M. Desimone, A. C. Porter, M. S. Garet, K. S. Yoon, B. F. Birman, *Educ. Eval. Policy Anal.* **24**, 81 (2002).
5. M. S. Garet, A. C. Porter, L. Desimone, B. F. Birman, K. S. Yoon, *Am. Educ. Res. J.* **38**, 915 (2001).
6. J. A. Supovitz, H. M. Turner, *J. Res. Sci. Teach.* **37**, 963 (2000).
7. W. R. Penuel, B. J. Fishman, R. Yamaguchi, L. P. Gallagher, *Am. Educ. Res. J.* **44**, 921 (2007).
8. J. G. Freeman, R. W. Marx, L. Cimellaro, *J. Sci. Teach. Educ.* **15**, 111 (2004).
9. E. A. Davis, J. S. Krajcik, *Educ. Res.* **34**, 3 (2005).
10. R. M. Schneider, J. Krajcik, *J. Sci. Teach. Educ.* **13**, 221 (2002).
11. W. R. Penuel, L. P. Gallagher, S. Moorthy, *Am. Educ. Res. J.* **48**, 996 (2011).
12. E. R. Banilower, D. J. Heck, I. R. Weiss, *J. Res. Sci. Teach.* **44**, 375 (2007).
13. M. S. Garet et al., *The Impact of Two Professional Development Interventions on Early Reading Instruction and Achievement* (Institute of Education Sciences, Washington, DC, 2008).
14. M. S. Garet et al., *Middle School Mathematics Professional Development Impact Study* (Institute of Education Sciences, Washington, DC, 2011).
15. J. I. Heller, K. R. Daehler, N. Wong, M. Shinohara, L. W. Miratrix, *J. Res. Sci. Teach.* **49**, 333 (2012).
16. K. J. Roth et al., *J. Res. Sci. Teach.* **48**, 117 (2011).
17. J. Osborne, S. Simon, A. Christodoulou, C. Howell-Richardson, K. Richardson, *J. Res. Sci. Teach.* **50**, 315 (2013).
18. J. Grigg, K. A. Kelly, A. Gamoran, G. D. Borman, *Educ. Eval. Policy Anal.* **35**, 38 (2013).
19. A. Lakshmanan, B. P. Heath, A. Perlmutter, M. Elder, *J. Res. Sci. Teach.* **48**, 534 (2011).
20. R. F. Elmore, M. L. Forman, E. L. Stosich, C. Bocala, *The Internal Coherence Assessment Protocol & Developmental Framework* (Strategic Education Research Project, Cambridge, MA, 2012).
21. A. S. Bryk, P. B. Sebring, E. Allensworth, S. Luppescu, J. Q. Easton, *Organizing Schools for Improvement* (Univ. of Chicago Press, Chicago IL, 2010).
22. S. Aud et al., *Condition of Education 2010* (National Center for Education Statistics, Washington, DC, 2010).
23. P. Cuevas, O. Lee, J. Hart, R. Deaktor, *J. Res. Sci. Teach.* **42**, 337 (2005).
24. J. A. Luft, S. Wong, I. Ortega, *The National Science Teacher Association (NSTA) State of Science Education Survey* (NSTA, Arlington, VA, 2009).
25. R. Lara-Alecio et al., *J. Res. Sci. Teach.* **49**, 987 (2012).
26. C. Dede, D. J. Ketelut, P. Whitehouse, L. Breit, E. M. McCloskey, *J. Teach. Educ.* **60**, 8 (2009).
27. O. Lee, R. Deaktor, C. Enders, J. Lambert, *J. Res. Sci. Teach.* **45**, 726 (2008).
28. R. W. Marx, P. C. Blumenfeld, J. S. Krajcik, E. Soloway, *Teach. Teach. Educ.* **14**, 33 (1998).

10.1126/science.1230725

PERSPECTIVE

A Business View on U.S. Education

Rick Stephens* and Mike Richey

Business leaders depend on an education system capable of providing a workforce able to compete in a global marketplace. As a partner in education reform, the business community advocates for an increased focus on helping schools connect students more effectively to the world of work through hands-on problem-solving activities and practical experiences.

Government leaders recognize that being poorly educated is tied to unemployment, poverty, crime rates, and spiraling social services costs. Parents recognize that a quality education is key to their children's future. Teachers and educators view their students' success as key to their life's work. Business leaders seek a workforce that can compete in a global marketplace at a time when many jobs cannot be filled for a lack of qualified workers (1). Thirty years ago, the U.S. National Commission on Excellence in Education stated that "Our society and its educational institutions seem to have lost sight of the basic purposes of schooling and of the high expectations and disciplined effort needed to attain them" (2). Although modest progress has been made since then, the world has been changing faster than its education systems. We believe business leaders would argue that many reform efforts have not yet focused on the right issues.

In the past 2 years, the Boeing Company has hired 33,000 new employees, and we observe two important phenomena. First, these new employees, from those lacking a high school diploma to those with a college degree, are generally quite good at using digital tools. Second, many seem to have rarely been put in situations where they have had to use their knowledge and skills to create a product of value. Those we hire have lots of ideas and can be savvy about many aspects of information technology. But those who haven't had to relate to a real-world experience of building something, or worked in a situation where failure is a real possibility, will generally need to spend considerable time on remedial training activities. For example, Boeing must now spend 13 weeks training employees for the same manufacturing jobs that used to require half the time; but even then, the employee often remains weak in the skills needed to manipulate materials effectively.

We believe the above observation is common among business leaders, spanning many business sectors. Although more is required from an education than preparing students for the world of work, such findings have important implications

for education reform. Today, when students are not in the classroom, they may spend up to 12 or more hours per day using some form of electronic media, whether sending text messages by cell phone, watching TV, or engrossed in using a computer, with yet unclear impacts on learning and problem solving (3). While greater use of technology in and out of the classroom holds promise and should be explored, we consider it unlikely that computers and simulations will fully substitute for real experiences in which students manipulate materials and engage in problem-solving in groups.

The classroom therefore needs to be a place where students spend a substantial amount of time applying what they learn to solving relevant problems that are appropriate to their age. As stated by the Ministry of Education of Singapore, teaching should "encourage a spirit of innovation and enterprise in students, and nurture intellectual curiosity, passion, and courage to try new and untested routes (4)."

We suggest that businesses focus on helping schools connect students more effectively to the world of work. In the United States, new synergies with industry arise from the recently adopted Common Core standards in English Language Arts and Mathematics, plus the forthcoming Next Generation Science Standards (NGSS), which stress in-class discourse, explanation, argumentation, reading and writing informational text, and active problem-solving (5, 6) [see page 276 in (7)].

The NGSS aim to incorporate real experiences into the classroom. But, as highlighted elsewhere in this issue [see page 320 in (8)], if we end up using inexpensive standardized assessments that mainly test for factual recall, the standards movement will simply prolong our failure.

Retired executive of The Boeing Company, 100 North Riverside, Chicago, IL 60606-1596, USA.

*Corresponding author. E-mail: rickstephens@me.com

The Education Task Force of the Illinois Business Roundtable has concluded that “the business community, in partnership with political and education leaders, must play a significant leadership role in education reform (9).” Recognizing that “education improvement is a marathon and not a hundred-yard dash” and that “education reform needs to be a collaborative, not adversarial, effort,” we have helped to create the Career Pathways Program, where businesses are working with the Illinois State Board of Education to bring practical, experience-based curricula into the classroom that can help ensure that students are either job ready or college ready when they graduate from high school (10).

Nationally, academic and business leaders have come together in efforts to create effective learning environments outside of the classroom experience. For example, FIRST robotics competitions were founded on the premise that students can succeed

when they compete, not just in a simulated game environment, but in the real world where there are winners and losers (11). These types of activities need to become part of formal schooling, not merely optional add-ons.

In summary, what can business do? First, be a strong advocate for exposing students to more hands-on problem-solving activities in the classroom. Second, help to provide scarce resources by increasing sponsorship of programs that engage students in such activities. Third, create more internship opportunities that allow students to be exposed to real-world work environments and directly learn what jobs are about. Fourth, support initiatives to question, and limit, the television, computers, and electronic games that can divert students’ time and attention away from other world experiences needed for future success. We believe that professional success today and in the future is more likely for those who have practical

experience, work well with others, build strong relationships, and are able to think and do, not just look up things on the Internet.

References

1. *New York Times*, “A Sea of Job Seekers, but Some Companies Are Not Getting Any Bids,” 23 June 2012.
2. *A Nation at Risk: The Imperative for Educational Reform* (National Commission on Excellence in Education, Washington, DC, 1983).
3. www.bls.gov/opub/mlr/2008/11/art4full.pdf
4. www3.moe.edu.sg/bluesky/tllm.htm
5. Common Core, www.corestandards.org/
6. NGSS, www.nextgenscience.org/
7. E. K. Stage, H. Asturias, T. Cheuk, P. A. Daro, S. B. Hampton, *Science* **340**, 276 (2013).
8. J. W. Pellegrino, *Science* **340**, 320 (2013).
9. The Education Task Force of the Illinois Business Roundtable, www.illinoisbusinessroundtable.com/education2011.htm
10. The Career Pathways Program, www.illinoisworknet.com
11. FIRST Robotics, www.usfirst.org/

10.1126/science.1230728

REVIEW

Outside the Pipeline: Reimagining Science Education for Nonscientists

Noah Weeth Feinstein,^{1*} Sue Allen,² Edgar Jenkins³

Educational policy increasingly emphasizes knowledge and skills for the preprofessional “science pipeline” rather than helping students use science in daily life. We synthesize research on public engagement with science to develop a research-based plan for cultivating competent outsiders: nonscientists who can access and make sense of science relevant to their lives. Schools should help students access and interpret the science they need in response to specific practical problems, judge the credibility of scientific claims based on both evidence and institutional cues, and cultivate deep amateur involvement in science.

For half a century, the world’s wealthiest countries have asked their education systems to teach science to all students, including those who will not go on to scientific careers (1). Under slogans such as “science literacy” and “science for all,” schools have attempted to prepare all students to make sense of science in daily life. With the exception of modest and isolated gains in conceptual knowledge (2), it is not clear that these campaigns have enhanced people’s ability to function in a world where conflicting health advice clutters the Internet, research is filtered through political screens, and the media strips context from scientific claims.

These results should provoke renewed interest in the relationship between science educa-

tion and public engagement with science and the pursuit of more fruitful forms of science literacy. Instead, many scientists and policy-makers are turning their attention away from the role of science in daily life and advocating a greater focus on the so-called “pipeline”: preprofessional education that delivers science-ready students to colleges and universities (3). Even crusaders for science literacy take for granted that scientific training—of the same sort that prepares students for scientific practice—will help nonscientists navigate fields as diverse as personal health, politics, the economy, leisure, and employment (1, 4, 5). There is little empirical evidence to support this assumption. On the other hand, a growing number of studies show untrained citizens engaging with science in adaptive ways (6). These citizens, whom Feinstein refers to as “competent outsiders” (7), identify relevant pieces of science and understand their local or personal implications without relying on school-based knowledge of particular scientific methods or concepts (6, 8).

How can education help more people act like competent outsiders? We synthesize evidence to develop a research-based plan for cultivating competent outsiders: nonscientists who can access and interpret the science most relevant to their lives. We reconsider established goals of science education in light of three central findings about public engagement with science and discuss implications for research and practice.

How People Interact with Science

Research shows that different groups interpret science differently (6, 9–12). An Alzheimer’s advocacy group, biotech investment firm, and religious coalition may all be interested in stem cell research, but different motivations underlie their interest and shape their engagement. Social, cultural, and demographic differences influence how people engage with science, both in school (13) and out (6, 11). For example, communications researchers have identified six demographically distinct groups of Americans who respond to news about climate change in predictable, group-specific ways (11). Local knowledge and experience, such as the history of tension between rural residents and a nuclear power plant (14), can play an important role. There are many different “publics” for science, each with different concerns and resources for making sense of the world.

To complicate matters, science is not a single, uniform thing. Science education places particular value on experimentation, but some fields rely on observational data or simulations, whereas others are devoted to theoretical inquiry. Even closely related fields can diverge on important matters, such as the validity of research methods or the nature of acceptable evidence (15). Nonscientists typically interact with specific manifestations of science rather than “science” as a whole (6, 12, 16). Although scientists may agree on abstract principles (such as hypothesis-testing) and

¹Departments of Curriculum & Instruction and Community & Environmental Sociology, University of Wisconsin–Madison, 225 North Mills Street, Madison, WI 53706, USA. ²Allen and Associates, Newcastle, ME 04553, USA. ³Centre for Studies in Science and Mathematics Education, University of Leeds, Leeds LS2 9JT, UK.

*Corresponding author. E-mail: nfeinstein@wisc.edu

methodological heuristics (such as model-building), the science of climate modeling is very different from the science of clinical trials, and understanding the family resemblance between them may not help a layperson make sense of evidence.

This leads to perhaps the most important finding: Although some people are interested in science for its own sake, many engage with science in response to situation-specific needs and tend to be interested in science only insofar as it helps them solve their problems (6–10, 12). Thus, a mother seeking therapies for her autistic son may explore research literature, but she is not attempting to understand that literature from a scientist's perspective. Instead, she labors to integrate what she learns with her knowledge of local services and her first-hand understanding of her child (16). Context shapes the process of engagement, and scientific principles take on different significance in different contexts, where they are laden with social and ethical implications (8, 10, 12). The challenges of daily life are what cognitive scientists call ill-structured problems, defined in personal and practical terms. Scientific understanding may contribute to the solution, but will rarely be the entire solution. It is important to be realistic about the sort of understanding people seek—and need—to make decisions (17).

Reconsidering the Goals of Science Education

These findings about public engagement with science strain the credibility of established approaches to science education. Scientists, educators, and policy-makers claim that science education is useful (1–3, 5), but what use is it to know a canonical collection of facts or an allegedly generic scientific method if people engage with specific pieces of science in highly contextualized ways? Can education prepare students for the deep idiosyncrasy of daily life? Evidence on public engagement indicates that students should still know science, think scientifically, and appreciate science—but it may be necessary to reconsider the established interpretation of these goals and the strategies used to achieve them.

Knowing Science: From Knowing the Textbook to Accessing the Science You Need

No set of scientific concepts and principles, no matter how carefully chosen, will be sufficient preparation for future engagement with science. This is a consequence of the unpredictable path of scientific progress, shifting social and political demands on scientific knowledge, and the variety of contexts and motives that drive public engagement. Even if it were possible to predict the future of science, one could never anticipate how science will ripple through the diverse future lives of students (4, 9, 12). Yet prior knowledge is only one piece of the sense-making apparatus that people use in their encounters with science. When reading a scientific article, a person draws on prior knowledge to interpret the text, but she does not stop when she is unfamiliar with a concept or uncertain of implications; she looks up the concept online, cross-

references a second article, discusses the matter with friends, and seeks out complementary expertise (6, 16, 18). People employ social and material resources to solve problems and answer questions, and encounters with science are an impetus for new learning as well as tests of prior knowledge.

Resources alone do not guarantee fruitful engagement with science: The same literature that reveals impressive sense-making ability among laypeople also reveals failures, frustrations, and uneven competence (6, 12, 16). Science education should prepare more students to access and interpret scientific knowledge at the time and in the context of need. Public engagement with science is not simply the application of scientific knowledge; it requires translating a daily problem into scientific terms and reconstructing the scientific answer amid the constraints of daily life (6, 12, 16). A rural resident worried about pesticide contamination must learn to express his concerns in questions that science can answer: What pesticides, at what doses, are most harmful? Are there reliable tests for pesticides in my children's air or water? These questions lead to answers that must then be translated back into local reality: Who will help me test my water? What can I do to mitigate the risks? The decision-making process incorporates both scientific and nonscientific information.

One promising approach for preparing students to succeed in such circumstances is Problem-Based Learning (PBL), which confronts students with ill-structured challenges, asking them to extend their existing knowledge and develop concrete solutions (19). PBL can produce durable knowledge gains and foster metacognitive skills that underlie self-directed learning, although researchers have yet to identify which features of PBL contribute most to learning (19, 20). Developed in medical schools, PBL needs further validation in kindergarten through grade 12 (K-12) settings, but it shares features with other promising pedagogies specific to K-12, such as Science-Technology-Society (STS) and Place-Based Education (21, 22). All of these mimic public engagement with science by making the problem a focus for learning, allowing students to develop complex questions and test the adequacy of their answers, and, in many cases, using authentic social and practical problems that cannot be defined in purely scientific terms.

Fundamental problems of research and practice must be addressed before these pedagogies can be used to greatest effect. Little is known about using them together, or over time, to help students recognize when and how science is relevant. Students frequently struggle to apply what they have learned in one specific context to another; on the other hand, teaching generic problem-solving skills appears to have limited value (23, 24). Finding the right level of specificity, and honing strategies to connect multiple learning episodes, are problems of longstanding interest to researchers and educators (23, 25). Educators and researchers should work together to adapt problem-focused pedagogies for a

broad range of audiences, develop appropriate assessments, and—critically—find the most productive balance between these strategies and other means of presenting disciplinary science content.

Thinking Scientifically: From Practicing Science to Judging Scientific Claims

“Thinking scientifically” has been interpreted in many ways, from the trial-and-error experimentalism of early progressives to the scientific method dogma of the post-war era and the more flexible (if also more vague) idealism of scientific inquiry (1, 5). In the United States, the forthcoming Next Generation Science Standards decompose scientific inquiry into distinct but interconnected “scientific practices” such as modeling, argumentation from evidence, and communication of results. This is an important step forward. It rejects the empirically dubious notion of a single scientific method, offers greater specificity than most inquiry frameworks, and better represents the collaborative and iterative aspects of scientific work (5).

Yet the scientific practices approach still emphasizes the scientist's “insider” perspective, neglecting cues that help outsiders make informed judgments. Nonscientists rarely need to replicate the iterative processes of systematic research, and literature suggests that it is difficult to transfer principles of research design, learned in disciplinary contexts, to the highly variable circumstances of daily life (23, 24). On the other hand, nonscientists do need to judge the trustworthiness and local validity of putatively scientific claims. Studies show that competent outsiders make sophisticated judgments about the credibility of scientific claims based on cues like professional reputation, publication venue, institutional affiliation, and potential conflicts of interest, even when they do not understand technical nuances of experimental design or laboratory technique (6, 8, 10). In one classic sociological study, local knowledge and historical context, combined with direct observation of scientists in the field, helped farmers make sophisticated counterarguments to government-sponsored studies when their grazing lands were contaminated by radioactive fallout (14). Studies have emphasized the importance of trust, reputational networks, and heuristic reasoning in judgment and decision-making (10, 26).

Science education could do far more to help people judge scientific claims based on the information available to them. This is important given the decline in dedicated science journalism at for-profit news organizations; increasingly, citizens are turning to the Internet, with its variable quality and political motivations, for science news (10). Lessons that focus on scientific argumentation and communication are part of the solution because they help students understand how scientists evaluate evidence and how research is packaged for presentation to various audiences (5, 27). Yet even this shortchanges the histories, institutions, and norms that contribute to the reliability of scientific knowledge. Competent outsiders appreciate the socio-political nuances

of “how science really works,” including scientific credentials, the role of peer review in research funding and publication, and the differing perspectives of the many types of research organizations (8, 10). They can navigate the changing world of popular science media, recognizing signs of source bias and understanding the difference between journalistic and scientific accounts of research (10).

This material can be dry and inaccessible when presented out of context, but promising pedagogies offer platforms for examining scientific credibility in realistic contexts. In Socio-Scientific Issue Discussions (SSID), students engage in structured conversation about a science-inflected social problem, with the goal of uncovering epistemic and ethical nuances at the interface of science and daily life (28). Other strategies focus on the creation and interpretation of science texts, ranging from research articles to popular science journalism (29, 30). These pedagogies must be refined to reveal the social and institutional structures of science. Although both address the credibility and usefulness of different sources, and both provide apt venues for exploring issues of institutional trust, work is needed to develop a systematic and developmentally appropriate set of scaffolds for learning about topics such as peer review and conflicts of interest.

Appreciating Science: From Positive Feelings to Deep and Durable Involvement

Most adults in high-income countries express mild but consistent interest in scientific topics (31), but formal education may have little to do with this: A substantial fraction of students in those same countries lose interest in science as they progress through school (32). Schools may lag behind informal learning environments in their ability to inspire and develop students’ interest in science (33). Older, top-down mechanisms for public engagement are being joined by science cafes, participatory science games, and maker spaces (community-oriented places that foster collaboration and resource-sharing in small-scale design and fabrication projects). Children and adults may connect with science through “citizen science” and “professional-amateur” communities dedicated to phenology, astronomy, and even molecular biology (18). People who interact with science through these platforms do so for widely varying reasons connected to personal interest and social identity (18, 33). In this rich and dynamic context, how and why should schools continue to foster appreciation of science?

Research suggests that deep, personal interest in some field of science provides motivation for future interactions, even with science in unrelated fields. Students who pursue their own science-related interests have a stronger sense of their ability to learn science in the future (33) and are less likely to lose interest over time (34). Their involvement in personally or socially meaningful science-related activities can lead to learning experiences that resemble project-based learning and socio-scientific issue discussions (35). When students find a particular

scientific topic compelling, they seek experiences that prepare them for future encounters with science. Knowledgeable amateurs can become powerful resources for their communities (9, 18, 33).

Schools wishing to develop deep and durable involvement in science should embrace the diversity of student interests—a challenge for educational systems accustomed to pushing everyone toward the same goal. Three pathways hold promise. First, educators can use the flexibility provided by project- and place-based pedagogies to help students identify and develop individual interests and expertise. Second, schools can pursue partnerships with museums, which excel at sparking curiosity, and with afterschool clubs and community organizations, which provide flexible spaces for ongoing exploration (33). Third, educators can integrate science-based games and citizen science engines like FoldIt and GalaxyZoo into their curricula. Researchers should develop efficient ways to track the development of lasting student interests and identify productive ways to integrate informal experiences and game-based technologies into schools and classrooms (33).

Implications

On the way to becoming competent outsiders, students should learn to (i) access and interpret science in the context of complex, real-world problems; (ii) judge the credibility of scientific claims based on both social and epistemic cues; and (iii) cultivate deep and durable involvement in science, even when it takes them away from the formal curriculum. In practice, this means moving strategies such as PBL, SSID, and interest-driven student exploration from the pedagogical margins to the center. Allotting more time and resources to these strategies will result in a better balance between pre-professional science education and science education for nonscientists; given that PBL is used in a range of academically rigorous contexts, it may pay dividends for future scientists as well.

These strategies are works in progress. Too few studies investigate the challenges of moving from practical problems to scientific questions and integrating science back into practical solutions. Too few studies identify skills needed to reverse-engineer

a robust and coherent knowledge structure using real-world resources. Educational research on scientific epistemology neglects the diverse circumstances in which people encounter scientific claims, as well as the social and institutional knowledge that contributes to evaluating those claims. Research on deep and durable involvement in science is in its infancy; although there are portraits of success in games-based learning and informal science education, practice outstrips research. There is an urgent need to understand how and why these settings succeed (and fail) to transform attitudes, motivation, and identities.

Educators should not wait for these questions to be answered. Useful research requires real-world cases to study, and it is educators who will do much of the work of adapting project-based learning and other strategies to diverse K-12 settings. Predictable challenges loom: School schedules, parent expectations, and high-stakes testing militate against pedagogies that sacrifice short-term knowledge gains for complex skills, increased motivation, and a narrower but longer-lasting body of knowledge. Teachers and administrators should work together to clear space for pilot programs that test and demonstrate the value of these approaches. It may be most effective to deploy them as solutions to other widely acknowledged problems. For example, STS education produces motivational gains among students who are less likely to enroll in science courses (21), whereas PBL has found early champions in gifted education, with students who may have exhausted their local course offerings (19). Pilot programs conducted in these contexts can serve as beachheads for broader adoption.

Scientists may be allies or adversaries in reform. Some have played a decisive role in pedagogical and curricular progress, whereas others have defended the battlements for the established facts-and-principles approach (1). The scientific pipeline dominates educational discourse today, but it is those outside the pipeline who would benefit most from reform. Serving their needs requires a different sort of activism, and new attention to evidence about how, when, and why people interact with science.

Grand Challenges

Help students explore the personal relevance of science and integrate scientific knowledge into complex practical solutions. Teaching science in this way requires a focus on authentic problems that often cannot be defined in purely scientific terms.

Develop students’ understanding of the social and institutional basis of scientific credibility. Science education should empower students to make reasonable judgments about the trustworthiness and local validity of scientific claims, even when they don’t have deep background knowledge or access to expertise.

Enable students to build on their own enduring, science-related interests. Schooling that fosters the development of idiosyncratic interests, habitual curiosity, and lifelong science-related hobbies will strengthen students’ motivation and confidence in future learning experiences.

References and Notes

1. G. DeBoer, *A History of Ideas in Science Education* (Teachers College Press, New York, 1991).
2. J. Miller, *Curator* **53**, 191 (2010).
3. *Rising Above the Gathering Storm* (National Academies, Washington, DC, 2007).
4. S. Miller, *Public Underst. Sci.* **10**, 115 (2001).
5. National Research Council, *A Framework for Science Education* (National Academies Press, Washington, DC, 2012).
6. B. Irwin, Wynne, Eds. *Misunderstanding Science?* (Cambridge Univ. Press, Cambridge, 1996).
7. N. Feinstein, *Sci. Educ.* **95**, 168 (2011).
8. J. Ryder, *Stud. Sci. Educ.* **36**, 1 (2001).
9. W.-M. Roth, A. Calabrese Barton, *Rethinking Scientific Literacy* (Routledge Falmer, New York, 2004).
10. T. Bubela et al., *Nat. Biotechnol.* **27**, 514 (2009).
11. A. Leiserowitz, E. Maibach, C. Roser-Renouf, J. Hmielowski, "Global Warming's Six Americas, March 2012 & November 2011" (Yale Project on Climate Change Communication, Yale Univ. and George Mason Univ., New Haven, CT, 2012); <http://environment.yale.edu/climate/files/Six-Americas-March-2012.pdf>.
12. D. Layton, E. Jenkins, S. Macgill, A. Davey, *Inarticulate Science?* (Studies in Education Ltd, Driffield, UK, 1993).
13. O. Lee, C. A. Buxton, *Diversity and Equity in Science Education* (Teachers College Press, New York, 2010).
14. B. Wynne, *Public Underst. Sci.* **1**, 281 (1992).
15. P. Galison, *Image and Logic* (Univ. Chicago Press, Chicago, 1997).
16. N. W. Feinstein, *Public Underst. Sci.* **2012**, 0963662512455296 (2012).
17. R. Fernandes, H. A. Simon, *Policy Sci.* **32**, 225 (1999).
18. R. Bonney et al., *Public Participation in Scientific Research: Defining the Field and Assessing Its Potential for Informal Science Education* (Center for Advancement of Informal Science Education, Washington, DC, 2009); <http://caise.insci.org/uploads/docs/PPSR%20report%20FINAL.pdf>.
19. C. E. Hmelo-Silver; Hmelo-Silver, *Educ. Psychol. Rev.* **16**, 235 (2004).
20. C. Wirkala, D. Kuhn, *Am. Educ. Res. J.* **48**, 1157 (2011).
21. J. Bennett, F. Lubben, S. Hogarth, *Sci. Educ.* **91**, 347 (2007).
22. S. Semken, C. B. Freeman, *Sci. Educ.* **92**, 1042 (2006).
23. J. D. Bransford, A. L. Brown, R. R. Cocking, Eds., *How People Learn* (National Academies Press, Washington, DC, 2000).
24. D. Schwartz, J. D. Bransford, D. Sears, in *Transfer of Learning from a Modern Multidisciplinary Perspective*, J. Mestre, Ed. (Information Age, Charlotte, 2005), pp. 1–51.
25. R. L. Goldstone, S. B. Day, *Educ. Psychol.* **47**, 149 (2012).
26. D. Kahnemann, *Thinking, Fast and Slow* (Farrar, Straus, and Giroux, New York, 2011).
27. J. Osborne, *Science* **328**, 463 (2010).
28. T. D. Sadler, D. L. Ziedler, *J. Res. Sci. Teach.* **46**, 909 (2009).
29. J. S. Krajcik, L. M. Sutherland, *Science* **328**, 456 (2010).
30. S. P. Norris, L. Phillips, *Sci. Educ.* **87**, 224 (2003).
31. National Science Board, *Science and Engineering Indicators 2012* (National Science Foundation, Arlington, VA, 2012).
32. J. Osborne, S. Simon, S. Collins, *Int. J. Sci. Educ.* **25**, 1049 (2003).
33. National Research Council, *Learning Science in Informal Environments* (National Academies Press, Washington, DC, 2007).
34. D. Vedder-Weiss, D. Fortus, *J. Res. Sci. Teach.* **49**, 1057 (2012).
35. J. H. Falk, M. Storksdieck, L. D. Dierking, *Public Underst. Sci.* **16**, 455 (2007).

Acknowledgments: The authors thank T. Keller and J. Rudolph for comments.

10.1126/science.1230855

REVIEW

Generating Improvement Through Research and Development in Education Systems

M. Suzanne Donovan

To effectively address problems in education, research must be shaped around a problem of practice. Reorienting research and development in this way must overcome three obstacles. First, the incentive system for university researchers must be changed to reward research on problems of practice. Second, the contexts must be created that will allow the complexity of problems of practice to be understood and addressed by interdisciplinary teams of researchers, practitioners, and education designers. And third, meaningful experimentation must become acceptable in school systems in order to develop a better understanding of how to effectively stimulate and support the desired changes.

The connection between research and practice in the field of education has been weak (1). The “knowing-doing gap” is lamented in other fields as well, including business management (2) and medicine (3). But it is difficult to find a parallel in education to the design of digital devices by technology companies that have fundamentally changed how we go about our daily lives, or the application of biomedical research to save lives in extreme circumstances.

How might we make use of research knowledge to pursue new possibilities and design new tools and processes to improve education? The fact that other sectors have made major strides in some regards, yet struggle to reliably incorporate verified improvements into practice, highlights two distinct challenges. One is a design

challenge: When research informs designs that solve a problem from the point of view of the users, barriers to change disintegrate (4). Doctors, for example, use magnetic resonance imaging because it allows them to see what they otherwise cannot without risky or invasive procedures. And people have changed routine behavior enthusiastically when given access to technological innovations such as smartphones and Internet search engines. But when an innovation requires that people change their behavior to achieve goals others have set—to get hospital physicians to wash their hands or to use checklists that reduce errors (5), or to motivate teachers to engage students in classroom discourse rather than to teach through lectures (6)—it is an implementation challenge (7). The challenges are interrelated: Greater success at designing for the user implies fewer implementation barriers. If it is made easier for doctors to disinfect their hands, they are more likely to do so. But school sys-

tems and hospitals are intended to serve the goals of others, making improvements in practice desirable whether or not the user embraces the change. Research and development (R&D) will therefore need to address both design and implementation challenges.

While the task is far from simple, its components can be described in the most basic terms. They are (i) identifying the right problem, (ii) developing effective solutions, and (iii) getting effective solutions to spread.

Identifying the Right Problem

Scientific research can be driven either by theory or by problems of practice. Research that contributes to both falls into “Pasteur’s quadrant” (8). The National Institutes of Health and the National Science Foundation support programs of “translational research” intended to make advances in research knowledge usable for practice (9, 10). The term “translational” suggests that the required knowledge is in hand. It needs only to be put into the language of practice.

Rarely do problems of education practitioners map neatly onto areas of scientific research, however. Even in the case of pasteurization, translation would be a mischaracterization. Pasteur’s scientific breakthrough came with a commission to work on a practical problem: the spoiling of wine (11). The problem-solving research did not end with the realization that bacteria cause the spoiling, nor with the evidence that heat could be used to destroy bacteria. The heating process changes the end product—whether wine or milk—affecting taste, appearance, and digestibility (12). It took decades of work on the time and temperature of heating and cooling to develop the process of pasteurization that revolutionized the delivery of milk (13). The translation metaphor conceals the way in which research that solves problems of practice is shaped and

Strategic Education Research Partnership Institute, Washington, DC 20005, USA.

E-mail: sdonovan@serpinstitute.org

disciplined by the problem to produce usable and desirable solutions.

A National Research Council Committee that explored the weak relationship between research and practice in education (1) concluded that, in contrast to medicine and agriculture, education researchers have few opportunities to identify the specific problems of practice that can serve as productive starting points for programs of research and development. For example, research illuminates the influence of social and emotional factors on learning (14). If students think effort matters, they are more likely to persevere when schoolwork becomes difficult than if they believe intelligence is fixed (15), and they will be exposed as having too little. And if a student believes he or she is expected to do poorly—a condition that can be manipulated in experiments—performance declines (16). The importance of these findings is clear, but how they might be effectively incorporated into practice is not.

One opportunity for intervention in the classroom arises when students give incorrect answers (17). At that moment a teacher can further explore the student's thinking, signaling both the expectation that struggling will produce learning, and that the student is capable of thinking further about the problem. However, when teachers call on another student or provide the answer themselves, their responses are consistent with a belief that "some people have it and some people don't."

Identification of opportunities like this will remain infrequent unless researchers spend time in practice settings more routinely in order to observe and articulate problems of practice for which research knowledge can contribute potential solutions. A first grand challenge, then, is to shift the incentive structures in universities and funding agencies that privilege theoretical research and its translation over problem-solving research in practice settings.

Finding Effective Solutions

Will asking the right questions guarantee effective solutions? If by "effective" we mean solutions that work in the real world, the answer is no. Efficacy trials attempt to control interactions with the system and those in it in order to create fidelity. But this is not a test of a system's ability to achieve those outcomes under normal conditions (18). The many ways in which a system acts to reject or undermine an intervention must itself be treated as a set of problems to be solved (19). The treatment of adolescents hospitalized for cystic fibrosis provides an example, and bears all the marks of an education problem, right down to the resistant adolescents (20). Cystic fibrosis inpatients require airway clearance therapy (ACT) four times per day, done in accordance with best practice. In a hospital in Cincinnati, best practice was used only 10% of the time, and at baseline the four sessions were done on only 41% of patient days in the hospital. Education and personal

coaching of respiratory therapists, as well as salary incentives to following best practice, failed.

Identifying the obstacles to full implementation resulted in a list of 30. Patient behaviors on the list, familiar to any educator, include defiance, lack of knowledge, immaturity complex, inadequate parental support, and absence of consequences. Other obstacles shared with education systems include lack of coordination among professionals, legitimate activities of lesser importance, inadequate staffing, and supply shortages.

A multidisciplinary team tackled high-leverage component problems. Psychologists used well-researched approaches for changing the behavior of adolescents, including the introduction of signed contracts, performance monitoring, and providing small rewards for follow-through. Schedules were made at intake with ACT sessions as the first priority. "Improvement research" protocols (21) were used to test components of practice. The incidence of best practice rose to 73%, and the number of patient days in which the recommended ACT number was achieved rose from 41% sustainably to 68% (20).

The initial identification of "best practice" is only a starting point for improvement. Designing a sustainable solution required varied expertise and inputs from medical practitioners, psychologists, administrators, and supply managers. And leadership was needed to galvanize the cross-functional teams toward a common purpose (21). A second grand challenge thus emerges: Can we create the settings in education where multidisciplinary researchers, education designers, and education practitioners are led and supported to follow the contours of problems in order to identify systemically sustainable solutions?

Getting Solutions to Spread

If a solution is designed to work effectively in a complex system under normal circumstances, can we assume that it will spread to other systems (22) or even be maintained in the initial site (18, 23)? The answer is no. David Cutler poses the question provocatively: "Why do people and institutions not do things that are so obviously in their self-interest, even when they want to do so?" (24). Where one begins to look for an answer will vary by disciplinary orientation. Economists look first at incentives. The incentives lens, when focused on school systems, leads to policies that give or withhold monetary rewards for changes in system performance or practices. A focus on teachers leads to recommendations such as pay for performance or abolishing tenure. And some economists have proposed that students be paid to do well (25).

Sociologists look for answers in the culture and social norms of organizations. People are more likely to do what others around them do, as evidenced in smoking, eating, and exercising habits (26). In education settings, social norms that reject changes or prohibit questioning of a professional's practice are impediments

to change (19, 27). In medicine, checklists with demonstrated ability to reduce adverse incidents in surgery were used more effectively when social norms were shifted so that nurses who routinely defer to doctors were charged with calling out a checklist violation (5).

Psychologists look for answers in the individual's cognitive and emotional processes (28). External incentives and organizational norms are part of an individual's calculus, but influences also include personal goals, orientations, knowledge, and resources (29), as well as expectations of outcomes based on beliefs about one's ability to succeed and about reactions from others (30). Control over the conditions for behavior change (31), and the specificity of an individual's planning, also matter (32).

In a comprehensive study of psychological theories of behavior change, researchers in the field of "implementation science" have attempted to understand which theoretical explanation for individual behavior has the greatest predictive value with regard to medical practitioners' decisions (33). Several of the theories, as measured in the study, had no predictive value. Despite the breadth of the variables included, the best-performing theories explained between 25 and 42.6% of the variance in intended behavior but only 2.4 to 6.3% of actual behavior (33).

Confronted with so broad an array of potential contributors to behavior change, and such striking evidence of failure to scale improvements, what can be done? Invest further in the scholarly endeavor of understanding professional behavior, as implementation scientists are attempting? Deepen the knowledge base on how education organizations use research knowledge, and how they might do so more effectively? Engage in problem-solving research and development, and learn about human and organizational behavior in the process of experimenting with changing it (24)? All would no doubt be valuable. But if the ultimate goal is to improve practice, then problem-solving R&D has two features to recommend it. First, the lag time between the initial investment in research and change in practice will be shorter if the new knowledge is generated as a by-product of testing interventions in practice. This is particularly important if practitioners are to see value in research. Second, there is evidence that the contributors to behavior privileged by economists, sociologists, and psychologists are all important in some contexts. When either the importance of context or its variation is low, theoretical research may be an efficient route to generating knowledge that is relevant to practice. This would be the case if the goal were to understand how students grasp the concept of quantity, for example. But when both the importance and the variety of contexts are high, as with shifts in professional practice, generating knowledge by intervening and observing what happens is likely to be more

illuminating. If, for example, teachers shift more willingly to inquiry teaching when they have routine opportunities to problem-solve with their colleagues, when the materials are replenished by a designated staff member, and when parents are given weekly updates about science activities, it is difficult to imagine that such contextual factors could be identified without experimenting in practice settings. Moving from practice to theory is likely to be more fruitful than the reverse. But it will require that meaningful experimentation in education settings become acceptable. This is the third of the grand challenges.

Supporting Experimentation in Education Settings

The concept of experimentation does not sit well in systems that are highly accountable to the public. Parents would need to be persuaded that their children's education was not being jeopardized. And school system administrators and teachers who have many constituencies, none of which clamor for experimentation, would need to have the time, resources, flexibility, and incentives to engage in experimentation.

While each of the grand challenges is daunting on its own, they become oddly less so as a set. If, for example, there were practice sites where R&D collaborations were supported routinely (challenge 2), then researchers may find time spent working in those settings to be more productive and more supportive of goals related to quality and number of publications (challenge 1). And consistent financial support for such sites would provide an incentive to overcome an aversion among educators to experimentation, allow for the development of a different culture that is consistent with the designation as an R&D site, and provide the potential for a shift in the perception of parents regarding experimentation (challenge 3)—just as hospitals associated with experimental research and development are often sought out because they are on the leading edge.

There are signs of movement. Increasingly, researchers are partnering with school districts to tackle problems of practice (34), and funding agencies are beginning to award grants for research partnerships (35). Two efforts are under way to establish organizational structures to support research in practice settings. The Strategic Education Research Partnership (SERP), incubated at the National Research Council, is creating R&D “field sites” with school districts in which multidisciplinary teams of researchers and education designers are recruited to work on problems of practice identified by the districts (36). The Carnegie Foundation has created a model for “Networked Improvement Communities (NICs)” involving an organizing “hub” and partner organization “spokes” (37). NICs address multiple drivers of change related to a core problem of practice. Both models show promise for addressing design and implementation challenges. Federal funding agencies are creating new grant competitions that emphasize research-practice partnerships. But in relation to the size of the education sector, these efforts are miniscule.

The rapid pace of technological change has created new possibilities for education, at a time of emerging consensus on the critical importance of improving our education system. To seize the moment, we will need to think and work differently. There will be no “silver bullets” that will transform education systems from the outside—not in the form of new standards, assessments, programs, or technologies. While these changes are important, they will come up against inevitable implementation barriers. But if we create the organizational capacity for researchers and design experts to work with practitioners inside the system, we could potentially change the outcome. It will be possible to identify opportunities to use technology to solve problems that students, teachers, and administrators are trying to solve and to follow the contours of the implementation problems that arise as new standards, practices, and assessments are introduced. New knowledge

and new technologies will have their best chance at improving school systems if the nation invests in that capacity.

References and Notes

1. M. Donovan, A. Wigdor, C. Snow, Eds., *Strategic Education Research Partnership. Committee on a Strategic Education Research Partnership* (The National Academies Press, Washington, DC, 2003).
2. J. Pfeffer, R. I. Sutton, *The Knowing-Doing Gap: How Smart Companies Turn Knowledge into Action* (Harvard Business School Press, Boston, MA, 2000).
3. Institute of Medicine, *Crossing the Quality Chasm: A New Health System for the Twenty-First Century* (National Academy Press, Washington, DC, 2001).
4. <http://dschool.stanford.edu/wp-content/uploads/2011/03/BootcampBootleg2010v2SLIM.pdf>
5. A. Gawande, *The Checklist Manifesto: How to Get Things Right* (Picador, New York, 2011).
6. M. M. Kennedy, *Inside Teaching: How Classroom Life Undermines Reform* (Harvard Univ. Press, Cambridge, MA, 2005).
7. M. P. Eccles, B. S. Mittman, *Implement. Sci.* **1**, 1 (2006).
8. D. E. Stokes, *Pasteur's Quadrant: Basic Science and Technological Innovation* (Brookings Institution Press, Washington DC, 1997).
9. <http://commonfund.nih.gov/clinicalresearch/overview-translational.aspx>
10. www.nsf.gov/funding/pgm_summ.jsp?piims_id=503524&org=EHR
11. S. Feinstein, *Louis Pasteur: The Father of Microbiology* (Enslow, Berkeley Heights, NJ, 2008).
12. M. J. Lewis, N. Heppell, *Continuous Thermal Processing of Foods: Pasteurization and UHT Sterilization* (Aspen, Gaithersburg, MA, 2000).
13. V. H. Holsinger, K. T. Rajkowski, J. R. Stabel, *Rev. Sci. Tech. Off. Int. Epiz* **16**, 441 (1997).
14. D. S. Yeager, G. M. Walton, *Rev. Educ. Res.* **81**, 267 (2011).
15. C. S. Dweck, *Self-Theories: Their Role in Motivation, Resonality and Development* (Taylor and Francis/ Psychology Press, Philadelphia, 1999).
16. C. M. Steele, *Am. Psychol.* **24**, 645 (2003).
17. Observations made in a series of classroom “walk-throughs” by research collaborators in a SERP field site, Winter 2012.
18. M. W. McLaughlin, D. Mitra, *J. Educ. Change* **2**, 301 (2001).
19. M. K. Stein, C. E. Coburn, *Am. J. Educ.* **114**, 583 (2008).
20. M. P. Ernst et al., *J. Pediatr.* **117**, 14 (2010).
21. M. Jain, L. Miller, D. Belt, D. King, D. M. Berwick, *Qual. Saf. Health Care* **15**, 235 (2006).
22. S. Stringfield, A. Datnow, *Educ. Urban Soc.* **30**, 269 (1998).
23. C. E. Coburn, *Educ. Res.* **32**, 3 (2003).
24. D. M. Cutler, Why Don't People and Institutions Do What They Know They Should? *American Economic Association, Ten Years and Beyond: Economists Answer NSF's Call for Long-Term Research Agendas*. Available at Social Science Research Network, <http://ssrn.com/abstract=1888580> or <http://dx.doi.org/10.2139/ssrn.1888580>.
25. R. G. Fryer, *Q. J. Econ.* **126**, 1755 (2011).
26. N. H. Gottlieb, J. A. Baker, *Soc. Sci. Med.* **22**, 915 (1986).
27. W. Penuel, M. Riehl, A. Krause, K. Frank, *Teach. Coll. Rec.* **111**, 124 (2009).
28. S. Michie et al., “Psychological Theory” Group, *Qual. Saf. Health Care* **14**, 26 (2005).
29. A. H. Schoenfeld, *ZDM* **43**, 457 (2011).
30. A. Bandura, *Social Foundations of Thought and Action: A Social Cognitive Theory* (Prentice-Hall, Englewood Cliffs, NJ, 1986).
31. D. J. Terry, J. E. O'Leary, *Br. J. Soc. Psychol.* **34**, 199 (1995).
32. A. E. Walker et al., *BMC Health Serv. Res.* **3**, 22 (2003).
33. M. P. Eccles et al., *Implement. Sci.* **7**, 99 (2012).
34. W. R. Penuel, B. J. Fishman, *J. Res. Sci. Teach.* **49**, 281 (2012).
35. http://ies.ed.gov/funding/pdf/2013_84305H.pdf
36. M. S. Donovan, C. Snow, P. Daro, www.serp-institute.org/2013/about/principles-of-serp-collaborations/.
37. A. S. Bryk, L. M. Gomez, A. Grunow, (2011), *Frontiers in Sociology of Education*, M. T. Hallinan, Eds. (Springer, New York, 2011), pp. 127–162.

10.1126/science.1236180

Grand Challenges

Shift incentives to encourage education research on the real problems of practice as they exist in school settings. The current incentive structures in universities and in agencies that fund education support theoretical research and its “translation” for practice. Those incentives must shift to encourage research on the problems of practice in practice settings.

Create a set of school districts where long-standing, multidisciplinary teams work together to identify effective improvements. Because the problems of practice are multi-dimensional, addressing them effectively will require the creation of settings in which teams of researchers, practitioners, and education designers are supported to follow the contours of a problem in order to identify effective improvements.

Create a culture within school systems that allows for meaningful experimentation. Because contextual factors are critical to the effective scaling of improvements, experimentation in real school contexts is critical. This will require a major shift from the belief that experimentation is inappropriate in schools systems to the belief that it provides new opportunities for the success of education professionals and the students they serve.

REVIEW

Proficiency in Science: Assessment Challenges and Opportunities

James W. Pellegrino

Proficiency in science is being defined through performance expectations that intertwine science practices, cross-cutting concepts, and core content knowledge. These descriptions of what it means to know and do science pose challenges for assessment design and use, whether at the classroom instructional level or the system level for monitoring the progress of science education. There are systematic ways to approach assessment development that can address design challenges, as well as examples of the application of such principles in science assessment. This Review considers challenges and opportunities that exist for design and use of assessments that can support science teaching and learning consistent with a contemporary view of what it means to be proficient in science.

We face extraordinary promise for the future of science learning, juxtaposed with substantial challenges in achieving the vision of what it means to be proficient in science (1). Among those challenges are determining how the proficiency of our students will be assessed relative to that vision and doing so in ways that support, rather than inhibit, teaching and learning. Educational assessments ought to be statements about what scientists, educators, policy-makers, and parents want students to learn and become. It is well established that what we choose to assess will end up being the focus of instruction. So, it is critical that science assessments, both external and internal to the classroom, best represent the proficiencies we desire. This Review argues that much of what is needed to effectively assess science learning, either at the classroom level or for purposes of system monitoring, has yet to be created and that design and implementation challenges are substantial. Even so, there are promising cases from which to learn and build (2).

Shared Perspectives on Proficiency

A disjuncture exists between students' knowledge of science facts and procedures, as assessed by typical achievement tests, and their understanding of how that knowledge can be applied through the practices of scientific reasoning, argumentation, and inquiry (3, 4). This problem is recognized in reports spanning kindergarten (K) to grade 16+ (K-16+) that simultaneously present a consistent description of what proficiency in science should be (1, 5-11). Seldom has such a consistent message been sent as to the need for change in what we expect students to know and be able to do in science, how science should be taught, and how it should be assessed. The emergent definition of proficiency is perhaps most clearly expressed in three major elements of the U.S. National Research Council (NRC) Frame-

work for K-12 Science Education (1): (i) core or "big" ideas within disciplinary areas, (ii) practices of scientific and engineering reasoning, and (iii) cross-cutting concepts. Collectively they define what it means to know science, not as separate elements but as intertwined aspects of knowledge and understanding [see also (12)]. It is not just the description of each and their intersection that matters but also that the meaning of proficiency is realized through performance expectations about what students at various levels of educational experience should know and be able to do. These statements move beyond vague terms such as "know" and "understand" to more specific statements like "analyze," "compare," "explain," "argue," "represent," "predict," "model," etc. in which the practices of science are wrapped around and integrated with core content. Educators and researchers are also recognizing that proficiency develops over time and increases in sophistication and power as the product of coherent systems of curriculum, instruction, and assessment.

The virtue of such a view is that science educators are poised to better define the outcomes desired from their instructional efforts, which in turn guides the forms of assessment that can help them know whether their students are attaining the desired objectives, as well as how they might better assist them along the way. It is very important for the science education community, and policy-makers and the public more broadly, to develop a shared perspective on what constitutes high-quality and valid science assessments across K-16+ if assessments are to support teaching and learning and attainment of the desired science education outcomes.

Proficiency, Performance Expectations, and Assessment Design Challenges

The NRC Framework uses the logic of progressions to describe students' developing proficiency in three intertwined domains—practices, cross-cutting concepts, and core ideas—in a coherent way across grades K through 12. The framework builds in the idea of a progression of student understanding across the grades by specifying grade-band end-

point targets at grades 2, 5, 8, and 12 for each component of each core idea. The framework also provides sketches of possible progressions for acquiring each practice or cross-cutting concept but does not indicate the expectations at any particular grade level. The Next Generation Science Standards (NGSS) (7) build on these suggestions and include tables that define what each practice might encompass and the expected uses of each cross-cutting concept for students at each grade level.

This integrated perspective of what it means to know science suggests that assessment should help determine where a student can be placed along a sequence of progressively more "scientific" understandings of a given core idea that by definition includes successively more sophisticated applications of practices and cross-cutting concepts. This is an unfamiliar idea in the realm of science assessments, which have more often been viewed as simply measuring whether students know particular grade-level content. It means that assessments must strive to be sensitive both to grade-level appropriate performances and to intermediate performances that may be appropriate at somewhat lower or higher grade levels. This is particularly important for the design of assessment materials and resources that can be used in classrooms to support instruction.

The NRC Framework states that assessment tasks must be designed to gather evidence of students' ability to apply the practices and their understanding of the cross-cutting concepts in the contexts of problems that also require them to draw on their understanding of specific disciplinary ideas. It suggests using a model put forward in Science Standards for College Success (13) by expressing standards in terms of performance expectations. The organization Achieve and its partners in NGSS development have elaborated these guidelines into standards that are clarified by descriptions of the ways in which students at each grade are expected to apply both the practices and the cross-cutting concepts and of the knowledge they are expected to have of the core ideas. The NGSS appear as sets of performance expectations related to a particular aspect of a core disciplinary idea (see the draft example in Fig. 1). Each performance expectation asks students to use a specific practice in the context of a specific element of the disciplinary knowledge relevant to the particular aspect of the core idea. Across the set of expectations at a given grade level, each practice and cross-cutting concept appears with multiple standards.

Performance expectations also may include boundary statements that identify limits to the level of understanding or context appropriate for a grade level and clarification statements that offer additional detail and examples. But standards and performance expectations, even as explicated in the NGSS, lack sufficient detail to create an assessment.

From NRC Frameworks, Standards, and Performance Expectations to Assessments

The design of valid and reliable science assessments hinges on elements that include but are not

Learning Sciences Research Institute, University of Illinois at Chicago, Chicago, IL 60607, USA.

E-mail: pellegjw@uic.edu

restricted to what is articulated in disciplinary frameworks and standards such as those illustrated above (14, 15). In the design of assessment items and tasks related to performance expectations, one needs to also consider (i) the kinds of conceptual models and evidence in which we expect students to engage, (ii) grade-level appropriate contexts for assessing performance expectations, (iii) options for task design features (e.g., computer-based simulations or animations, paper-pencil writing and drawing) and which of these are essential for eliciting students' ideas about the performance expectation, and (iv) the types of evidence that will reveal levels of student understanding and skill.

Assessment involves evidentiary reasoning (14), so it has proven useful to be more systematic in framing assessment development as an evidence-centered design process (ECD) [e.g., (15, 16)]. The process starts by defining the claims that one wants to be able to make about student proficiency—the

ways in which students are supposed to know and understand some particular aspect of a domain. Examples might include aspects of force and motion or heat and temperature. The most critical aspects of defining these are to be as precise as possible about what matters and to express this in the form of verbs such as “model,” “explain,” “predict,” etc. In essence, the performance expectations found in the NGSS are claims about student proficiency.

Claims about the student must be linked to forms of evidence that would support those claims. Evidence statements capture features of work products or performances that would give substance to the claims. This includes which features need to be present and how they are weighted—what matters most, least, or not at all. If evidence in support of a claim about a student's knowledge of the laws of motion is that the student can analyze a physical situation in terms of the forces acting on all the bodies, then the evidence might be drawing a free-body diagram with all

the forces labeled, including their magnitudes and directions.

The precision that comes from elaborating the claims and evidence statements pays off when it is time to design tasks or situations to provide the requisite evidence. Tasks are not designed or selected until it is clear what forms of evidence are needed to support the range of claims appropriate to a given assessment situation. The tasks need to provide necessary evidence and should allow students to show what they know in ways that are as unambiguous as possible with respect to what the performance implies about student knowledge and skill (17).

Science Assessment Example Cases

Given the relative newness of the NRC Framework, it is no surprise that comprehensive sets of assessment examples that align completely with the NGSS performance expectations do not exist. Many of the tasks that have been used for classroom

4-LS1 From Molecules to Organisms: Structures and processes

4-LS1 From Molecules to Organisms: Structures and processes

Students who demonstrate understanding can:

- 4-LS1-a. Use simple models to describe that plants and animals have major internal and external structures, including organs, that support survival, growth, behavior, and reproduction.** [Clarification Statement: Examples of structures include thorns, stems, roots, stamens, ovaries, heart, brain, skin, or bones.] [Assessment Boundary: Students are responsible for the overall functions of major structures, but the mechanisms of how they function within a system are not assessed. Students are not expected to memorize different types of structures but should be able to use information given.]
- 4-LS1-b. Design, test, and compare solutions that replace or enhance the function of an external animal structure necessary for survival.*** [Clarification Statement: Students might compare solutions for mobility based on the strength of different materials used.]
- 4-LS1-c. Construct models to describe that animals' senses receive different types of information from their environment, process the information in the brain, and respond to the information in different ways.** [Clarification Statement: Examples of models could be diagrams or analogies.] [Assessment Boundary: Students are not expected to know the mechanisms by which the brain stores and recalls information, nor the mechanisms of how sensory receptors function.]

The performance expectations above were developed using the following elements from the NRC document *A Framework for K-12 Science Education*:

Science and Engineering Practices

Developing and Using Models

Modeling in 3–5 builds on K–2 models and progresses to building and revising simple models and using models to represent events and design solutions.

- Develop a model using an analogy, example, or abstract representation to describe a scientific principle or design solution. (4-LS1-c)
- Identify limitations of models. (4-LS1-c)
- Use a simple model to test cause and effect relationships concerning the functioning of a proposed object, tool or process. (4-LS1-a)

Constructing Explanations and Designing Solutions

Constructing explanations and designing solutions in 3–5 builds on prior experiences in K–2 and progresses to the use of evidence in constructing multiple explanations and designing multiple solutions.

- Use evidence (e.g., measurements, observations, patterns) to construct a scientific explanation or design a solution to a problem. (4-LS1-b)
- Apply scientific knowledge to solve design problems. (4-LS1-b)

Obtaining, Evaluating, and Communicating Information

Obtaining, evaluating, and communicating information in 3–5 builds on K–2 and progresses to evaluating the merit and accuracy of ideas and methods.

- Compare and/or combine across complex texts and/or other reliable media to acquire appropriate scientific and/or technical information. (4-LS1-a)
- Use multiple sources to generate and communicate scientific and/or technical information orally and/or in written formats, including various forms of media and may include tables, diagrams, and charts. (4-LS1-a)

Disciplinary Core Ideas

LSA.A: Structure and Function

- Plants and animals have both internal and external structures that serve various functions in growth, survival behavior, and reproduction. (4-LS1-a), (4-LS1-b)

LS1.D: Information Processing

- Different sense receptors are specialized for particular kinds of information, which may be then processed and integrated by the animal's brain, with some information stored as memories. Animals are able to use their perceptions and memories to guide their actions. Some responses to information are instinctive—that is, animals' brains are organized so that they do not have to think about how to respond to certain stimuli. (4-LS1-c)

ETS1.C: Optimizing the Design Solution

- Different solutions need to be tested in order to determine which of them best solves the problem given the criteria and the constraints. (secondary to 4-LS1-b)

Cross-cutting Concepts

Structure and Function

- Substructures have shapes and parts that serve functions. (4-LS1-a), (4-LS1-b), (4-LS1-c)

Influence of Engineering, Technology, and Science on Society and the Natural World

- Engineers improve existing technologies or develop new ones to increase their benefits, decrease known risks, and meet societal demands. (4-LS1-b)

Fig. 1. Example of a set of possible grade 4 standards in life science from the January 2013 preliminary draft of the NGSS.

assessment, and those found in large-scale state, national, and international tests, focus primarily on science content or on aspects of scientific inquiry separate from content. With few exceptions, such assessments do not integrate core concepts and science practices in the ways intended by the NRC Framework or NGSS. Nevertheless, we can draw from example cases to illustrate what is needed, many of which have used an ECD approach to guide assessment design and validation. The examples are diverse in several ways—including the science content and practices represented, age and grade level, intended use (at the classroom, state, national or international level; whether the consequences of student performance have low or high stakes), as well as the innovative use of technology.

Classroom Instruction and Assessment

Several projects have developed assessments for use in classroom instruction with a particular emphasis on the integration of core science concepts with one or more science practices such as modeling; evidence-based explanation and argumentation; and/or the design of investigations to test hypotheses, analyze results, and construct explanations from data. Some of the clearest examples can be found in a volume edited by Alonzo and Gotwals (18) focused on learning progressions and in a special issue on assessment of the *Journal of Research on Science Teaching* (19). Additional examples include the SimScientists (20, 21), Science ASSISTments (22), and BioKids projects (23).

Several of these projects illustrate the feasibility of designing tasks and situations, whether in paper-and-pencil format or mediated via simulations embedded in technology, that challenge students to reason with and about core science concepts in life and physical science. They demonstrate ways to obtain forms of evidence that can serve multiple purposes, such as measurement of student proficiency as well as diagnosis of student thinking for instructional improvement. The SimScientists project has shown how assessment situations and tasks involving dynamic simulations of science phenomena can be built from a principled design process that supports classroom formative assessment as well as summative assessment in large-scale state programs (21).

National and International Large-Scale Assessment

Much of what students and teachers experience as science assessments is external to regular classroom instruction and comes in the form of large-scale state tests, for example, administered in response to the U.S. No Child Left Behind legislation. Although the quality of such state assessments varies, none approximates the performance expectations discussed in the NRC Framework and NGSS. In contrast, there are two large-scale assessment programs that more closely exemplify aspects of science proficiency that involve science

practices: the U.S. National Assessment of Educational Progress (NAEP) and the Programme for International Student Assessment (PISA).

The NAEP 2009 and 2011 assessments were constructed from a framework document that identified specific areas of content in the life, physical, and Earth and space sciences, as well as a set of science practices: (i) identifying science principles, (ii) using science principles, (iii) using scientific inquiry, and (iv) using technological design. Item types fell into two broad categories: selected-response items (such as multiple choice) and constructed-response items (such as short answer). To further probe students' abilities to combine their understanding with the investigative skills that reflect practices, a subset of the students completed hands-on performance or interactive computer tasks (3, 4, 24, 25). In contrast to NAEP, which is administered to 4th-, 8th-, and 12th-grade students, the PISA assessment is administered only to 15-year-olds. The most recent PISA science assessment results are based on a framework that includes science proficiencies that overlap with the science practices of the NRC Framework and NGSS, as well as aspects of the NAEP framework (26, 27).

What are especially important about both NAEP and PISA are the sets of simple and complex science assessment tasks that demand reasoning about science content as described in the NRC Framework and NGSS. Both assessment programs are a source of examples of the types of performances that align with the descriptions of proficiency discussed earlier. Neither NAEP nor PISA represent static assessment programs. Both undergo major revisions to the framework used to guide assessment design and task development, and both are increasingly moving to incor-

porate technology as a key aspect of task design and assessment of student performance. It is likely that the NAEP framework will be revised within the next decade, and work is already under way in revising the PISA science framework for 2015. Changes in both will ostensibly move in directions that even more closely align with the NRC Framework. Thus, both might constitute reasonable ways to monitor overall progress of science teaching and learning in U.S. classrooms in ways consistent with implementation of the NRC Framework and NGSS.

Advanced Placement (AP) Science

A contemporary approach to rethinking science proficiency can be found in the redesign of the AP courses and assessments for biology, chemistry, and physics (8, 9, 28, 29). The AP program offers college-level curricula to high school students. Starting in 2006, the College Board, which administers AP, with support from the U.S. National Science Foundation, initiated a process that started by redefining the focus, critical content, and science practices that should define proficiency at the end of each AP science course (30). This would then guide development of both a curriculum framework for each course as well as the high-stakes assessment often used by colleges for purposes of granting course credit and/or advanced course placement.

Using the complementary processes of backward design (31) and ECD, a framework was developed for each science discipline that is organized in terms of disciplinary big ideas, enduring understandings, and supporting knowledge as well as a set of seven science practices. This structure parallels that of the core ideas and science practices in the NRC Framework. Similar to what is advocated in the NRC Framework and

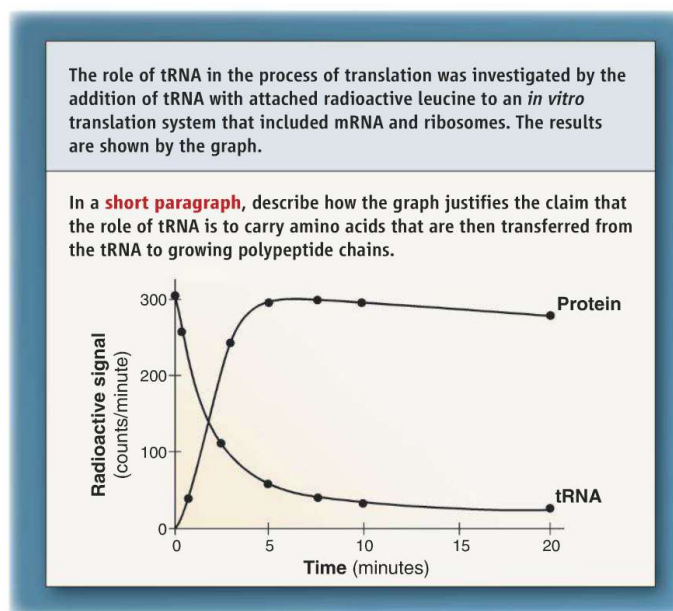


Fig. 2. A sample of a short constructed response item for the new AP biology exam.

Grand Challenges

Design valid and reliable assessments reflecting the integration of practices, cross-cutting concepts, and core ideas in science. The performance expectations of the Next Generation Science Standards (NGSS) pose significant challenges for assessment design. Considerable research and development will be needed to create and evaluate assessment tasks and situations that can provide adequate evidence of the proficiencies implied in the NGSS. This research must be carried out in instructional settings where students have had an adequate opportunity to construct the integrated knowledge envisioned by the National Research Council Framework and the NGSS.

Use assessment results to establish an empirical evidence base regarding progressions in science proficiency across K-12. Much of what is assumed in the NGSS regarding learning progressions needs to be validated through empirical research. This validation requires assessment tasks and situations that can be used across multiple age and grade bands so that we can determine how proficiency changes over time with appropriate instruction. The empirical results can then be used to support the design of more effective curriculum materials and instructional practices.

Build and test tools and information systems that help teachers effectively use assessments to promote learning in the classroom. For teachers to effectively implement assessment as part of their pedagogy, they need tools for presenting tasks and collecting and scoring student performance. They also need smart systems that provide actionable information about the meaning and implications of student performance relative to instruction and student learning. Such systems will need to be designed in collaboration with learning scientists and teachers to ensure their validity, usability, and utility.

realized in the NGSS, performance expectations or learning objectives were defined within each discipline to reflect the blending of core ideas with science practices. Through application of ECD, sets of claim-evidence pairs were elaborated in each science discipline to focus and support course instruction as well as development of assessment tasks for new AP exams.

The first of those exams will be given in May 2013 in biology, with chemistry to follow in 2014 and physics in 2015. To help teachers and students orient to the new course and exam, a wealth of materials including sample assessments were provided (32). To reflect the shift in focus demanded by integration of science practices with core content ideas, new item types were created, and a greater emphasis is being placed on constructed response questions. Figure 2 provides an example of a short constructed response item that involves the integration of conceptual knowledge with aspects of the practices.

AP science redesign is still a work in progress. Much remains to be determined about the quality and impact of the new exams on student learning and classroom instructional practice. But AP science instruction and assessment are changing in ways closely aligned with the perspective on science proficiency described earlier.

The Road Ahead

Assessment is a key element in the process of educational change and improvement. Done well, it can signify what we want students to know and be able to do and help educators create learning environments that support attainment of those objectives. Done poorly, it sends the wrong signals and skews teaching and learning. Our greatest danger may be a rush to turn the NGSS into sets of as-

essment tasks for use on high-stakes state accountability tests before we have adequately engaged in research, development, and validation of the range of tasks and tools needed to get the job done properly. Most especially we must ensure that teachers are given the time, support, and assessment tools to create instructional environments where their students have adequate opportunities to learn what is now expected of them.

References and Notes

- Committee on a Conceptual Framework for New K-12 Science Education Standards, Board on Science Education, National Research Council, *A Framework for K-12 Science Education: Practices, Crosscutting Concepts, and Core Ideas* (National Academies Press, Washington, DC, 2012).
- J. W. Pellegrino, *J. Res. Sci. Teach.* **49**, 831 (2012).
- National Center for Educational Statistics (NCES), *The Nation's Report Card: Science in Action: Hands-On and Interactive Computer Tasks From the 2009 Science Assessment* (NCES 2012-468, Institute of Education Sciences, U.S. Department of Education, Washington, DC, 2012).
- A. C. Fu, S. A. Raizen, R. J. Shavelson, *Science* **326**, 1637 (2009).
- Committee on Science Learning Kindergarten Through Eighth Grade, National Research Council, *Taking Science to School: Learning and Teaching Science in Grade K-8*, R. A. Duschl, H. A. Schweingruber, A. W. Shouse, Eds. (National Academies Press, Washington, DC, 2007).
- Committee on Learning Science in Informal Environments, National Research Council, *Learning Science in Informal Environments: People, Places, and Pursuits*, P. Bell, B. Lewenstein, A. W. Shouse, M. A. Feder, Eds. (National Academies Press, Washington DC, 2009).
- Achieve, *Next Generation Science Standards*, www.nextgenscience.org/.
- College Board, *The AP Biology Curriculum Framework 2012-2013* (College Board, New York, 2011).
- College Board, *The AP Chemistry Curriculum Framework 2013-2014* (College Board, New York, 2011).
- Committee on the Status, Contributions, and Future Directions of Discipline-Based Education Research, National Research Council, *Discipline-Based Education Research: Understanding and Improving Learning in Undergraduate Science and Engineering*, S. R. Singer, N. R. Nielsen, H. A. Schweingruber, Eds. (National Academies Press, Washington, DC, 2012).
- American Association of Medical Colleges, "MRS 5th comprehensive review of the Medical Colleges Admission Test (MCAT): Final MCAT recommendations" (2012), www.aamc.org/download/273766/data/finalmr5recommendations.pdf.
- H. Pratt et al., *The NSTA Reader's Guide to A Framework for K-12 Science Education, Expanded Edition: Practices, Crosscutting Concepts, and Core Ideas* [National Science Teachers Association (NSTA) Press, Arlington, VA, 2012].
- College Board, *Science College Board Standards for Success* (College Board, New York, 2009).
- Committee on the Foundations of Assessment, *Knowing What Students Know: The Science and Design of Educational Assessment*, J. W. Pellegrino, N. Chudowsky, R. Glaser, Eds. (National Academies Press, Washington, DC, 2001).
- R. J. Mislevy, G. Haertel, *Educ. Meas.* **25**, 6 (2006).
- R. J. Mislevy, M. M. Riconscente, in *Handbook of Test Development*, S. Downing, T. Haladyna, Eds. (Erlbaum, Mahwah, NJ, 2006), pp. 61-90.
- Although this discussion of a principled approach to assessment design has focused exclusively on specific tasks and situations, it is equally applicable to the design of an assemblage of tasks—an assessment or test that might be used for various purposes at the classroom level or beyond. Such a process also requires careful selection and assembly of tasks so that the desired inferences about student proficiency can be supported at the level of the overall instrument or its subparts. Thus, assessment design at multiple levels from separate tasks to larger instruments needs to always be approached as a principled design process in which the claims one wants to make about student proficiency are supported by sufficient forms of evidence carefully chosen to meet the intended purpose and use of the assessment results.
- A. C. Alonzo, A. W. Gotwals, Eds., *Learning Progressions in Science: Current Challenges and Future Directions* (Sense, Rotterdam, Netherlands, 2012).
- N. B. Songer, M. A. Ruiz-Primo, *J. Res. Sci. Teach.* **49**, 683 (2012).
- J. W. Pellegrino, E. S. Quellmalz, *J. Res. Technol. Educ.* **43**, 119 (2010).
- E. S. Quellmalz, M. J. Timms, M. G. Silberglitt, B. C. Buckley, *J. Res. Sci. Teach.* **49**, 363 (2012).
- J. Gobert, M. Sao Pedro, R. S. Baker, E. Toto, O. Montalvo, *J. Educ. Data Min.* **4**, 153 (2012).
- N. B. Songer, A. W. Gotwals, *J. Res. Sci. Teach.* **49**, 141 (2012).
- National Assessment Governing Board, *Science Framework for the 2009 National Assessment of Educational Progress* (National Center for Educational Statistics, Government Printing Office, Washington, DC, 2008).
- National Center for Educational Statistics, *The Nation's Report Card: Science 2011* (NCES 2012-465, Institute of Education Sciences, U.S. Department of Education, Washington, DC, 2012).
- Organization for Economic Cooperation and Development (OECD), *PISA 2009 Assessment Framework: Key Competencies in Reading, Mathematics and Science* (OECD, Paris, 2009).
- H. L. Fleischman, P. J. Hopstock, M. P. Pelczar, B. E. Shelley, *Highlights From PISA 2009: Performance of U.S. 15-Year-Old Students in Reading, Mathematics, and Science Literacy in an International Context* (NCES 2011-004, National Center for Education Statistics, U.S. Department of Education, Government Printing Office, Washington, DC, 2010).
- K. Huff, L. Steinberg, T. Matts, *Appl. Meas. Educ.* **23**, 310 (2010).
- W. B. Wood, *Science* **325**, 1627 (2009).
- College Board, *From Research to Practice: Redesigning AP Science Courses to Advance Science Literacy and Support Learning with Understanding*, final report submitted to the National Science Foundation for award number ESI-0525575 (2010).
- G. Wiggins, J. McTighe, *Understanding by Design* (Association for Supervision and Curriculum Development, Alexandria, VA, ed. 2, 2005).
- College Board, *AP Biology Course and Exam Description, Effective Fall 2012* (College Board, New York, 2012).

10.1126/science.1232065

Pervasive Externalities at the Population, Consumption, and Environment Nexus

Partha S. Dasgupta^{1,2*} and Paul R. Ehrlich^{3*}

Growing concerns that contemporary patterns of economic development are unsustainable have given rise to an extensive empirical literature on population growth, consumption increases, and our growing use of nature's products and services. However, far less has been done to reach a theoretical understanding of the socio-ecological processes at work at the population-consumption-environment nexus. In this Research Article, we highlight the ubiquity of externalities (which are the unaccounted for consequences for others, including future people) of decisions made by each of us on reproduction, consumption, and the use of our natural environment. Externalities, of which the "tragedy of the commons" remains the most widely discussed illustration, are a cause of inefficiency in the allocation of resources across space, time, and contingencies; in many situations, externalities accentuate inequity as well. Here, we identify and classify externalities in consumption and reproductive decisions and use of the natural environment so as to construct a unified theoretical framework for the study of data drawn from the nexus. We show that externalities at the nexus are not self-correcting in the marketplace. We also show that fundamental nonlinearities, built into several categories of externalities, amplify the socio-ecological processes operating at the nexus. Eliminating the externalities would, therefore, require urgent collective action at both local and global levels.

The presence of externalities in economic activity is widely acknowledged today; yet, the focus has been on externalities associated with humanity's use of the natural environment (1, 2). It is also widely appreciated that natural resources such as the global commons are underpriced, owing to those externalities. In contrast, consumption and reproductive externalities have been studied far less. The few empirical studies on this topic have found that those externalities can also be quantitatively important (3–5). In this paper, we characterize and classify reproductive and consumption externalities, relate them to environmental externalities, and show that they reinforce and are, in turn, reinforced by one another. For brevity we do not discuss production externalities, whose sources are, in any case, environment externalities.

Reproductive Externalities

Historical demographers have argued that the 17th-to-18th-century fertility transition in North-west Europe is traceable to a then-growing practice of establishing a new household upon marriage (6). By saving or transfer, couples had to have sufficient resources to establish and equip their new household. Those requirements led to

late marriages and meant that parents bore the cost of rearing their children. The total fertility rate (TFR) in England dropped to 4 in 1650 to 1700, when modern family-planning techniques were unknown and women were mostly illiterate.

In contrast, the TFR in sub-Saharan Africa remains over 5 today, and the region's population is expected to double to over 2 billion by 2050 (7). The average annual income in this region is currently 1200 international dollars (8). As sub-Saharan Africa has one of the highest TFRs in the world, yet is also one of the world's poorest regions, we have chosen to focus on aspects of African life that encourage high fertility and briefly compare these aspects with the changing situation elsewhere.

Pronatalist Institutions

Fosterage is commonplace in sub-Saharan Africa (9, 10). In parts of West Africa, up to half the children may be living with their nonparental kin at any given time (9). Fosterage is not adoption; it does not break ties between parents and children. The institution affords a form of mutual insurance protection in a region where formal insurance markets are thin. But under fosterage, the private cost of rearing one's own children is lower than the societal cost of child-rearing. An argument identical to the one establishing that unmanaged commons are overexploited can be used to show that the institution of fosterage encourages excessive fertility (11, 12).

Communal land tenure of the lineage social structure creates another problem of unmanaged commons, providing further incentive for men to

procreate. Moreover, because conjugal bonds are weak, fathers in sub-Saharan Africa bear even less of the costs of child-rearing than they do in regions where polygyny is not practiced. Frequently, there is no common budget for the man and woman. Descent in sub-Saharan Africa is, for the most part, patrilineal and residence is patrilocal (the Akan people of Ghana are an exception). Taken together, patrilineality, polygyny, communal land tenure, and the institution of fosterage give rise to a set of externalities that promote fertility (12). Those social practices may be weakening and would be expected to decline under urbanization, but, to date, they have persisted and carry with them a momentum from the past.

A report by the United Nations Development Programme indicates that empowerment of women, a desirable end in itself, is associated with lower TFRs (1). Early marriage, particularly in the Sahel and North Africa, contributes to some of the highest TFRs in the world. Very few girls (only 1 in 100 in Niger, for example) complete secondary school. Family planning is not subject to the play of "free markets"; it is biased by restrictive laws, widespread misinformation, and rules not based on evidence (13–16). The unmet need for family planning is substantial. For example, the proportion of women in Malawi who either want to delay their next baby or stop having children altogether, but who are not using contraception, is ~25%. Women who have greater autonomy are better equipped to surmount the many barriers that often prevent easy access to family planning. When the barriers are few, as in Indonesia, the use of contraception and the TFRs among the highest- and lowest-income quintiles are similar (15). When the barriers are numerous, as in the Philippines, the poor both have more children and a greater unmet need for family planning.

Access to family planning can be increased relatively quickly compared with other approaches to lowering TFRs, such as improving women's education (although the alternatives may be synergistic). Forty percent of the world's population (including countries with TFRs as high as 6 as

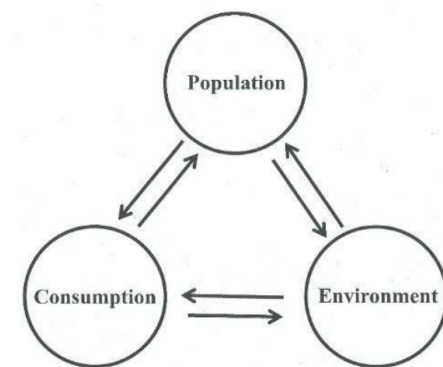


Fig. 1. Mutual influences amplified by externalities in the population-consumption-environment nexus.

¹Faculty of Economics, University of Cambridge, Cambridge CB3 9DD, UK. ²Sustainable Consumption Institute, University of Manchester, Manchester M13 9PL, UK. ³Centre for Conservation Biology, Department of Biology, Stanford University, Stanford, CA 94305, USA.

*Corresponding author. E-mail: partha.dasgupta@econ.cam.ac.uk (P.S.D.); pre@stanford.edu (P.R.E.)

recently as 50 years ago) now has TFRs that are at or below replacement level. The aggregate demand for environmental resources is, in part, a function of humanity's population size. Whether world population reaches 8 billion or 10 billion in 2050 and whether it reaches 15 billion or 17 billion in 2100 will depend on small differences in average family size, which could be highly influenced by rebuilding the focus on family planning (15, 16).

Conformity

In many spheres of life, traditional practices are perpetuated on account of a desire to conform (17–19). One's peer group matters, because it gives rise to another category of externalities. Procreation is not only a private matter but also a social signal, influenced by both family experiences and the cultural milieu. Conformism means that every household's desired family size is an increasing function of the average family size in the community. For instance, consider a society of N households. Let n_h be household h 's desired family size and n^* the average family size of the community to which the household belongs. If people were conformist, n_h would be a piecewise increasing function of n^* , to be written as $n_h(n^*)$. In turn, as n_h affects n^* , each household inflicts an externality on all other households. If N is large, each of the externalities is definitely small, but the sum over all households can be substantial. In equilibrium, the average of all the households' desired n_h values would equal n^* ; that is, $n_h = \frac{1}{N} \sum [n_h(n^*)] = n^*$ (18, 20).

To illustrate the structure of household preferences that display conformism, let $\mathbf{C}_h$ denote the quantities of goods and services h consumes (bold indicates that $\mathbf{C}_h$ is a vector of commodities), and let n_h be household size. We assume both are choice variables for household h (subject to budgetary and physiological constraints, of course). If $U_h(\mathbf{C}_h, n_h)$ is a numerical representation of h 's preferences over consumption bundles and family size, a simple form of conformist preferences is

$$U_h(\mathbf{C}_h, n_h) = V_h(\mathbf{C}_h, n_h) - \alpha_h(n_h - n^*)^2, \alpha_h > 0 \quad (1)$$

Here, V_h is an increasing function of $\mathbf{C}_h$ and, for small n_h , of n_h as well; α_h is a positive constant (21).

Whatever the basis of conformism, reproductive practices can persist even when their original purposes have disappeared, especially when misinformation about the safety of contraceptives is widely shared. So long as all others aim at large families (n^* is large), few households on their own would wish to deviate from the established practice, even when large family sizes prove to be an impediment to economic betterment, as they do in fragile ecosystems. But if a sufficiently large number of households were to restrict their fertility rates, others would do so as well (remember, n_h is an increasing function of n^*), improving their own prospects and those of their children. Thus conformism can harbor multiple, locally

stable equilibria (18). If we now embed the structure of preferences in Eq. 1 in a dynamic model of consumption, saving, and reproduction, it can be shown that demographic history in the model economy is path-dependent. Fertility transitions can then be interpreted as disequilibrium phenomena, where societies move from high- to low-fertility equilibria. We consider one possible pathway for such transitions (22).

People differ in the extent to which they conform (i.e., α_h values differ across households). Inevitably, there will be those who, for one reason or another, experiment, take risks, and refrain from joining the crowd. Educated women are among the first to make the move toward smaller families (23). These women are the tradition-breakers, and they would be the ones to trigger fertility transitions, as others follow in time. A rapid pathway is the influence that newspapers, radio, television, and now the Internet play in transmitting information about other lifestyles. The influence of cultural exchanges via the media was pursued in a survey of fertility transitions in recent history (4). An analysis of a natural experiment in India has found that fertility rates declined in step after staggered introductions of cable television across the Indian states (5).

Breakdown of the Commons and the Added Need for Labor

The poorest countries are, in large part, biomass-based subsistence economies. Much labor is needed, even for simple tasks. Moreover, households in these poor nations do not have access to the sources of domestic energy available to households in advanced industrial countries, nor do they have water on tap. In arid regions, water supply is often not close at hand, nor is wood fuel easily accessible when forests recede. The relative prices of alternative sources of energy and water faced by rural households in poor countries differ from those faced by households elsewhere. In addition to cultivating crops, caring for livestock, cooking food, and producing simple marketable products, household members have to spend as much as 5 hours per day fetching water and collecting fodder and wood (24). These are complementary activities and must be undertaken on a daily basis if the household is to survive. Labor productivity is low, not only because manufactured capital and human capital are scarce, but also because natural resources are scarce. From the age of 6, children in rural households in the poorest countries mind their siblings and domestic animals; fetch water; and collect wood, dung (in the Indian subcontinent), and fodder. For the most part, these children do not attend school. The educational facilities in the typical school are woefully inadequate, and parents need their children's labor. In semi-arid regions of the Indian subcontinent and sub-Saharan Africa, children between 10 and 15 years old routinely work at least as many hours as adult males.

The need for many hands can lead to a destructive spiral. The tipping point can be one or

more of many small changes. For example, in recent years mores that once regulated the use of the local resource base have changed in many parts of Asia and Africa. In the past, rural assets such as village ponds and water holes, threshing grounds, grazing fields, and woodlands were owned communally. Communities protected their local commons from overexploitation by relying on social norms, imposing fines for deviant behavior, and other means (24, 25). But the very process of modernization can erode traditional methods of control, increased urbanization and mobility being one pathway (24). Social norms are also endangered by civil strife and the usurpation of resources by landowners or the state (26). Also, rules practiced at the local level have not infrequently been overturned by central fiat. A number of states in the Sahel imposed rules that effectively destroyed communitarian management practices in the forests. Villages ceased to have authority to enforce sanctions on those who violated locally instituted rules of use. State authority turned local common-property resources into open-access resources (27). Whatever the cause, as social norms degrade, parents pass some of the costs of children on to the community by overexploiting the commons. This is yet another instance of a demographic externality, but, in this case, its origins lie in deterioration in the management of local common-property resources (18, 28).

Consumption Externalities

Consumption, both in total and in its composition, gives rise to externalities that have consequences for the present, as well as the future. Modern consumption practices that have dire environmental consequences, such as global climate disruption (externalities), have been much noted in the literature. Here, we focus on externalities that are internal to the drive we have for consumption. The choice of goods whose consumption creates the externalities we detail below depends on such factors as whether people are rich or poor, whether the poor in question live in poor countries or rich countries, and so on. But the particular drive for consumption we study here is a common human trait (17, 29).

As social animals, we are competitive as well as conformists. We want to attain status in our community in certain ways, yet we simultaneously strive to be like others in different ways.

Competitive Consumption

In his classic work on the Gilded Age, Veblen (30) spoke of "conspicuous consumption" so as to draw attention to types of consumption that serve as status symbols. Veblen's notion of status has been extended by social scientists to cover the tendency of people to try to outdo one another (31). Here, we call this drive "competitive consumption." To formalize, let $\mathbf{C}_h$, as before, denote a bundle of consumption goods in an N -person society. We now introduce a further good, denoted by X : the average consumption

level of which, in the community, is X^* (i.e., $X^* = {}_h\Sigma X_h/N$). If $U_h(\mathbf{C}_h, X_h, X^*)$ is a numerical representation of h 's preferences, consider the functional form (32)

$$U_h(\mathbf{C}_h, X_h, X^*) = V_h(\mathbf{C}_h, X_h/X^*) \quad (2)$$

Here, V_h is an increasing and concave function of each component of $\mathbf{C}_h$ and X_h/X^* (33).

Equation 2 reflects competitive preferences over X , which give rise to "rat races." In a free market, every household tries to beat all others in their consumption of X in a losing proposition, for no one is better off: In equilibrium everyone works harder so as to purchase and consume more X than they would if they all agreed to work less hard and consume less X (3, 32). If N is large, the effect of household h 's choice of X on X^* (the externality) is no doubt small, but the sum of the externalities can be shown to be non-negligible. The process creates a problem of the commons, albeit in a different sphere from common-property resources—structurally, however, they are the same. They both reflect aspects of the well-known prisoners' dilemma in game theory.

Competitive consumption (hosting expensive wedding ceremonies and birth celebrations) in subsistence economies may not have repercussions on the global environment, but it hinders the prospects the poor may have for escaping poverty (34). In rich societies, competitive consumption has further adverse consequences. For instance, automobiles make transportation simple and easy, but choices of the make and vehicle use are driven in many ways by the competitive urge (35). Moreover, car use is dependent on an underpriced resource: oil. If we add consumption habits into the equation and a growing complementary infrastructure (gas stations, expanded network of highways), we have a spiraling exploitation of natural capital and the environmental externalities that come allied to it (12, 15, 16). Environmental problems of this kind have certainly originated in today's rich countries, but the consumption of conspicuous, resource-intensive goods and services is known to be increasing in developing countries also (2, 36).

Conformism

Because people try to find ways to relate to one another, they also adopt patterns of consumption that reflect a desire for conformity, not competition (17, 29). Fads and fashions are brief occurrences, but conformist consumption can be persistent if it serves the need for social belonging—hence the term "relational goods" to characterize commodities that serve as an anchor for the desire and need to belong (37, 38).

To illustrate the character of social consumption, let $\mathbf{C}_h$, as before, denote a bundle of consumption goods. We now introduce an additional commodity, labeled X , which serves as a relational good. The average consumption level of X

in the community is X^* . If $U_h(\mathbf{C}_h, X_h, X^*)$ is a numerical representation of household h 's preferences, we return to the formulation of conformism in reproductive decisions to construct U_h as

$$U_h(\mathbf{C}_h, X_h, X^*) = V_h(\mathbf{C}_h, X_h) - \alpha_h(X_h - X^*)^2, \alpha_h > 0 \quad (3)$$

Here, V_h is an increasing function of each component of $\mathbf{C}_h$ and X_h (33).

Conformism leads to a different social dynamic from competitive consumption. The social environment that characterizes it is not a prisoners' dilemma but a "coordination game" (39). In contrast to a prisoners' dilemma, coordination games possess multiple equilibria, which can be ranked in terms of societal well-being. To confirm this in a simple manner, consider an extreme version of Eq. 3

$$U_h(\mathbf{C}_h, X_h, X^*) = V_h(\mathbf{C}_h), \text{ subject to } X_h = X^* \quad (4)$$

The idea underlying U_h in Eq. 4 is that some consumption choices (over X in this instance) reflect preferences only for consuming as others do, and not for any particular preference for the personal consumption of the goods in question (40).

Equation 4 makes clear that, as with conformism in the sphere of reproduction, a community may coordinate to settle on one of many alternative behavior patterns that are ranked identically by all individuals. Presumably, there would be equilibria that are more intensive than others in their use of underpriced natural capital. Consumption choices in the former type of goods inflict adverse externalities on other communities.

As the problem besetting the community is one of coordination, the collective assurance that people will choose a preferred level of X would suffice to improve consumption patterns. If each person can be persuaded to believe that others will reduce their consumption of X , every person will follow suit. One way of achieving that assurance would be to nudge one another to opt for a targeted alternative.

Environmental Externalities

Environmental externalities have been much discussed in the literature (2). Here, we focus on detrimental externalities. Two broad types may be contrasted: unidirectional and reciprocal. Under unidirectional externalities, one agent (or a set of agents) inflicts an externality on another (or others). Classic examples of unidirectional externalities involve pollutants transported downstream by wind and water. As is well known, the discharge of pollutants is excessive in the presence of such externalities.

Exports of primary products can come allied to domestic unidirectional externalities, a fact that has been rarely studied empirically. Logging in upstream watersheds gives rise to soil erosion and increased fluctuations in the supply of water downstream (the externalities). If compensation

were not paid to downstream farmers and fishermen for the loss of water and decimation of estuarine fish stocks, the export price of timber would be less than its social price (commonly known as "shadow price"). Therefore, the export would contain a hidden subsidy, amounting to a transfer of wealth to the importing country from farmers and fishermen in the exporting country.

Under reciprocal externalities, each party inflicts an externality on all others, as in the case of unmanaged common property resources. To manage the commons well means eliminating the externalities (25). Local communities have been known to manage spatially contained commons by the institution of norms of conduct and the imposition of fines and social sanctions (24, 25). Unfortunately, the same cannot be said in regard to the global commons. It is widely understood today that unmanaged commons are overexploited. Among these unmanaged commons, the atmosphere as a sink for carbon has been studied the most in recent years (1, 2), but the oceans consist of yet another class of global commons. Previously, we noted that local institutions have been known to degrade under pressure from markets and state intervention. Taking local and global commons together, nature's hidden subsidies have likely risen in recent decades.

Difficulties in Enacting Policies to Counter Externalities

Popular discourses on economic growth and development frequently assume that nature is a fixed, indestructible factor of production. Advanced textbooks on economic growth and development are based on a similar thesis. But nature consists of degradable resources. Agricultural land, forests, watersheds, fisheries, fresh water sources, estuaries, the atmosphere, and, more generally, ecosystems are self-regenerative capital assets, but they suffer from depletion or deterioration when they are overused or toxified.

Environmental externalities are pervasive because property rights to prominent classes of natural capital are difficult to enforce and, worse, challenging to define. One reason is their mobility: The wind blows, rivers flow, fish swim, deer flee, birds and insects fly, and even earthworms are known to move. Moreover, global climate change and the deteriorating state of the world's oceans indicate that substitution possibilities between produced capital and human capital, on one hand, and vital forms of natural capital, on the other, are limited (41). Official statistics on national income give the impression that the natural environment is of small importance (the share of agriculture in national income in the United States is only 2 to 3%, so there is no reason to worry), but official statistics are built on market prices, not prices that reflect the social worth of natural capital. If shadow prices were to be used in economic statistics, the decomposition of national income into its various components would look quite different (42, 43). The reproductive and consumption externalities we have outlined here

have a different origin, having to do with our psychological needs and drive.

Nonlinearities

Our account of reproductive and consumption externalities provides an explanation for why growth in population is a point of special concern in poor countries, whereas in rich countries with TFRs below 1.8, the focus should be wasteful consumption (15). We have shown not only that there is feedback between each node at the population-consumption-environment nexus (Fig. 1), but that externalities at each node amplify the feedback. Taken together, these factors create the unsustainable stresses on nature that have been recorded in recent years (44). The harmful effects of those stresses are made urgent by the presence of nonlinearities in the coupled processes at work. As the nonlinearities involve positive feedback, the stresses are yet further amplified and act more quickly (45, 46).

Nonlinearities are ubiquitous in the processes governing Earth; this is a central message of the environmental sciences (46). It is also known that competitive markets cannot exist in a world where production and consumption possibilities (including the use of natural capital) are subject to substantial nonlinearities (47). Modern economics has shown that nonlinearities are inevitable in social systems suffering from harmful environmental externalities (48). Hence, the externalities associated with competitive consumption generate yet another class of nonlinearities. Creating competitive markets for externalities as a way to eliminate their unintended consequences is, thus, not an option (47, 48). Nonmarket institutions are a necessary complement to customary markets. The creation of such institutions requires collective action among affected parties, but for that to come about, the parties need to be aware of the character of those externalities. This is beginning to happen in the case of a handful of nature's commons (1, 2)—for example, the global climate and the oceans—but, to date, there have been few attempts to estimate the magnitude of the reproductive and consumption externalities we have classified in this work.

Nonlinearities in socio-environmental systems are increasingly coupled with one another across space and time through trade in goods and services, which has aggravated problems by making individual risks overly correlated with one another (49). Because nonlinear systems can undergo regime shifts, the time it takes for a society to recognize that part of its system is near a tipping point until fully tipping into an unpalatable state is increasingly short. Currently, more than 45% of the 45 billion to 60 billion metric tons of carbon that are harnessed annually by terrestrial photosynthesis is being appropriated for human use (50). Due in large measure to that, 15 of the 24 major ecosystem services examined in the Millennium Ecosystem Assessment (44) were found to be either degraded or

currently subject to unsustainable use. Crude calculations suggest that if the 5.7 billion people in poor and middle-income countries were to match the consumption patterns of the 1.3 billion people in the rich world, at least two more Earths would be needed to support everyone on a sustained basis (51). The consensus among demographers is that world population will be 9.5 billion or more by 2050. Should that prove accurate, the demands made on Earth will prove to be even more unsustainable.

Technological Change

People are known to insist that technology can be relied on to come to humanity's aid (52), but new technologies do not come out of thin air. Innovators respond to incentives, and institutions matter. The underpricing of natural capital, for example, influences the direction of research and development, and the latter influences the character of technological change. Because nature's services are underpriced in the market, innovators have little reason to economize on their use. We should not be surprised when new technologies are rapacious in the use of natural capital. The cumulative adoption of such technologies and practices over the centuries has locked us into an infrastructure that will prove very hard to dislodge.

Evidence from the past tell us, however, that people have often responded successfully to growing economic stress by inventing new ways of doing things (53). After all, today's rich countries were poor not so long ago. But both evidence and the theoretical underpinning to that evidence tell us that successful response cannot be guaranteed. For example, in the face of population pressure in Bangladesh, small land-holders have periodically innovated so as to intensify agricultural production. However, this has resulted in imperceptible improvement in the standard of living and an increase in landless households (54), the latter probably owing to the prevalence of distress sales of land. By analogy, the image that comes to mind is of people walking up an escalator that is coming down at the same speed.

That image has been sharpened in recent years by the empirical discovery that the persistence of poverty and continued loss in biodiversity are closely connected in parts of sub-Saharan Africa (55). Moreover, recent archaeological studies showing that a number of societies in the distant past collapsed, owing to degradation of the local environmental-resource base, provide indirect evidence of the contemporary presence of alarming positive feedback within the population-consumption-environment nexus (56).

Conclusion

Although their magnitudes are likely to vary across societies, owing to differences in societal histories, institutions, customs, and ecologies, the reproductive and consumption externalities we have identified here share marked commonalities. Moreover, our analysis has uncovered reasons why technological innovations since the Industrial

Revolution have been rapacious in their reliance on natural capital. We have shown that the externalities studied in this paper are not self-correcting. Hence, our analysis points to a spiraling socio-environmental process, giving credence to the presumption that the pattern of contemporary economic growth is unsustainable.

References and Notes

1. United Nations Development Programme, *Human Development Report* (United Nations, New York, 2010).
2. World Bank, *World Development Report* (World Bank, Washington, DC, 2010).
3. J. Schor, *The Overspent American* (Basic Books, New York, 1998).
4. J. Bongaarts, S. C. Watkins, *Popul. Dev. Rev.* **22**, 639 (1996).
5. R. Jensen, E. Oster, *Q. J. Econ.* **124**, 1057 (2009).
6. J. Hajnal, *Popul. Dev. Rev.* **8**, 449 (1982).
7. Population Reference Bureau, *2012 World Population Data Sheet* (Population Reference Bureau, Washington DC, 2012).
8. An international dollar is a hypothetical currency that enables one to compare costs across countries with the help of a common reference point—namely, the U.S. dollar. So, an international dollar has the same purchasing power in all countries as the U.S. dollar has in the United States.
9. J. C. Caldwell, P. Caldwell, *Popul. Dev. Rev.* **13**, 409 (1987).
10. J. Illife, *Africans: A History of the Continent* (Cambridge Univ. Press, Cambridge, 2011).
11. By "excessive," we mean excessive from the point of view of the society in question.
12. P. Dasgupta, *Eur. Econ. Rev.* **44**, 619 (2000).
13. J. J. Speidel, D. C. Weiss, S. A. Ethelston, S. M. Gilbert, *Philos. Trans. R. Soc. London Ser. B* **364**, 3049 (2009).
14. M. Campbell, N. N. Sahin-Hodoglugil, M. Potts, *Stud. Fam. Plann.* **37**, 87 (2006).
15. The Royal Society, *People and the Planet* (Royal Society, London, 2012).
16. P. R. Ehrlich, A. H. Ehrlich, *Proc. R. Soc. London Ser. B* **280**, 20122845 (2013).
17. P. Bourdieu, *Distinction: A Social Critique of the Judgment of Taste* (Routledge and Kegan Paul, London, 1984).
18. P. Dasgupta, *An Inquiry into Well-Being and Destitution* (Clarendon Press, Oxford, 1993).
19. L. E. Blume, W. A. Brock, S. N. Durlauf, Y. M. Ioannides, in *Handbook of Social Economics*, J. Benhabib, M. O. Jackson, A. Basin, Eds. (North-Holland, Amsterdam, 2011), pp. 853–964.
20. If n_h is taken to be a continuous variable, as would be a reasonable assumption in the face of uncertainty in conceiving a child, equilibrium can be shown to exist.
21. The quadratic loss function in Eq. 1 has proven useful in econometric analysis of conformist behavior in consumption choices (19).
22. P. Dasgupta, in *Economic Theory for the Environment: Essays in Honour of Karl-Göran Mäler*, B. Kristrom, P. Dasgupta, K.-G. Lofgren, Eds. (Edward Elgar, Cheltenham, UK, 2002), pp. 118–128.
23. W. Lutz, S. K. C. Science **333**, 587 (2011).
24. N. S. Jodha, *Living on the Edge: Sustaining Agriculture and Community Resources in Fragile Environments* (Oxford Univ. Press, Delhi, 2001).
25. E. Ostrom, *Governing the Commons: The Evolution of Institutions for Collective Action* (Cambridge Univ. Press, Cambridge, 1990).
26. M. Gadgil, R. Guha, *This Fissured Land* (Oxford Univ. Press, Delhi, 1992).
27. R. Lopez, *World Bank Econ. Rev.* **12**, 105 (1998).
28. R. Aggarwal, S. Netanyahu, C. Ramano, *Environ. Dev. Econ.* **6**, 209 (2001).
29. M. Douglas, B. Isherwood, *The World of Goods: Towards an Anthropology of Consumption* (Routledge, London, 1996).
30. T. Veblen, *The Theory of the Leisure Class: An Economic Study of Institutions* (George Allen & Unwin, London, 1925).

31. R. Layard, *Happiness: Lessons from a New Science* (Penguin, New York, 2005).
32. K. J. Arrow, P. S. Dasgupta, *Econ. J.* **119**, F497 (2009).
33. Household h is assumed to choose C_h and X_h , subject to its budget constraint, having taken X^* as given.
34. A. Banerjee, E. Duflo, *Poor Economics* (Public Affairs, New York, 2011).
35. P. Kuhn, P. Kooreman, A. Soetevent, A. Kapteyn, *Am. Econ. Rev.* **101**, 2226 (2011).
36. Economists advocate taxes in rich countries to blunt the externalities associated with conspicuous consumption (31, 32), but the allied environmental externalities created by such patterns of consumption need to be taxed as well.
37. P. Donati, *Relational Sociology: A New Paradigm for the Social Sciences* (Routledge, London, 2011).
38. C. Sunstein, E. Ullmann-Margalit, *J. Polit. Philos.* **9**, 129 (2001).
39. G. J. Mailath, L. Samuelson, *Repeated Games and Reputations: Long-Run Relationships* (Oxford Univ. Press, New York, 2006).
40. The equality-constraint that defines U_h in Eq. 4 is the limiting case of U_h in Eq. 3 when α_h is infinity.
41. P. R. Ehrlich, L. H. Goulder, *Conserv. Biol.* **21**, 1145 (2007).
42. R. Repetto, W. Magrath, M. Wells, C. Beer, F. Rossini, *Wasting Assets: Natural Resources and the National Income Accounts* (World Resources Institute, Washington, DC, 1989).
43. K. J. Arrow, P. Dasgupta, L. H. Goulder, M. J. Mumford, K. Oleson, *Environ. Dev. Econ.* **17**, 317 (2012).
44. R. Hassan, R. Scholes, N. Ash, Eds., *Ecosystems and Well-Being: Current State and Trends* (Island Press, Washington, DC, 2005).
45. P. R. Ehrlich, J. P. Holdren, *Science* **171**, 1212 (1971).
46. W. Steffen et al., *Global Change and the Earth System* (Springer, Berlin, 2004).
47. K. J. Arrow, F. H. Hahn, *General Competitive Analysis* (Holden Day, San Francisco, 1971).
48. D. A. Starrett, *J. Econ. Theory* **4**, 180 (1972).
49. R. M. May, S. A. Levin, G. Sugihara, *Nature* **451**, 893 (2008).
50. P. M. Vitousek, P. R. Ehrlich, A. H. Ehrlich, P. A. Matson, *Bioscience* **36**, 368 (1986).
51. W. E. Rees, in *Encyclopedia of Biodiversity*, S. A. Levin, Ed. (Academic Press, San Diego, 2001), vol. 2, pp. 229–244.
52. M. Ridley, *The Rational Optimist: How Prosperity Evolves* (4th Estate, London, 2010).
53. E. Boserup, *Population and Technological Change* (Univ. of Chicago Press, Chicago, 1981).
54. B. L. Turner II, A. M. Shajaa Ali, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 14984 (1996).
55. C. B. Barrett, A. J. Travis, P. Dasgupta, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 13907 (2011).
56. K. W. Butzer, G. H. Endfield, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 3628 (2012).

Acknowledgments: We thank K. Arrow, M. Campbell, G. Daily, A. Dasgupta, N. Diamond-Smith, A. Ehrlich, L. Goulder, D. Karp, D. Kennedy, M. Potts, A. Ulph, and, most especially, the editors and three anonymous referees for their comments. P.R.E. thanks the Mertz Gilmore Foundation, the Winslow Foundation, and P. Bing and H. Bing for their support.

14 May 2012; accepted 28 February 2013
10.1126/science.1224664

REPORTS

Near-Field Interference for the Unidirectional Excitation of Electromagnetic Guided Modes

Francisco J. Rodríguez-Fortuño,^{1,2} Giuseppe Marino,¹ Pavel Ginzburg,¹ Daniel O'Connor,¹ Alejandro Martínez,² Gregory A. Wurtz,¹ Anatoly V. Zayats^{1*}

Wave interference is a fundamental manifestation of the superposition principle with numerous applications. Although in conventional optics, interference occurs between waves undergoing different phase advances during propagation, we show that the vectorial structure of the near field of an emitter is essential for controlling its radiation as it interferes with itself on interaction with a mediating object. We demonstrate that the near-field interference of a circularly polarized dipole results in the unidirectional excitation of guided electromagnetic modes in the near field, with no preferred far-field radiation direction. By mimicking the dipole with a single illuminated slit in a gold film, we measured unidirectional surface-plasmon excitation in a spatially symmetric structure. The surface wave direction is switchable with the polarization.

Interference is the cornerstone of various phenomena in nature, enabling numerous applications. In optics, it is intensively used in microscopy, stellar measurements, spectroscopy, and communication technologies, among many others, and is the basis behind the concepts of reflection, refraction, and light bending (1, 2). Typically, interference occurs as a result of the relative phase lag of different propagating waves. By contrast, nanophotonics—the branch of optics studying the interaction of light with subwavelength nanoscale structures—deals inherently with phenomena that occur via near-field interactions before appreciable phase lags can be ac-

cumulated (3). A radiationless form of interference in the near field (4) is behind new exciting applications such as the focusing of evanescent components to achieve subwavelength resolution in imaging (5–8). Near-field interference achieved through the full coherent control of the phase and amplitude of excitation light allows asymmetric spatial field localization (9, 10) and selection of propagation paths at intersections of waveguides (11).

We demonstrate near-field interference by considering a single source of radiation coupled to a mode with a vectorial structure of electromagnetic field. With an additional degree of freedom provided by the vectorial character of the field, control over the near-field interference can be achieved. We show that an elliptically polarized dipole can produce destructive or constructive interference of different evanescent components in its near field and, as a result, excite electromag-

netic modes in neighboring material structures, such as dielectric and plasmonic waveguides and diffraction gratings, with a controlled directionality of propagation.

Recently, several solutions for the directional excitation of surface plasmon polariton (SPP) waves (12, 13) have been proposed, including a backside-illuminated slit near a surface Bragg grating (14), the use of asymmetric slanted gratings (15) and chirped gratings (16), tilted-angle illumination of slits and gratings (17, 18), or the illumination of nearby, compact nanoantennas (19). Directional extraction of light from localized emitters with nanoantennas (20), emission directionality of thermal sources coupled to SPPs (21), and directional coupling to planar dielectric photonic waveguides (22) have been demonstrated. All of the above approaches have a resonant nature and rely on the careful selection of the wavelength and geometrical parameters of the structure, with the direction of excitation determined by an asymmetry of the structure and/or the incident light direction. Near-field dipolar interference provides a fundamentally different approach to unidirectional guided wave excitation with broadband (nonresonant) characteristics and the opportunity to achieve polarization-tunable directionality.

The phenomenon of unidirectional excitation can be understood by considering a dipole placed at a subwavelength distance d from a waveguide surface—for example, a dielectric slab or a single metal-dielectric interface (Fig. 1A). The high-spatial-frequency components of the dipole radiation allow the excitation of guided modes in the waveguide. The fundamental principle behind the effect of unidirectionality is the selective vectorial excitation of the electric field of the waveguide mode, dependent on the emitted polarization. At the location of the waveguide immediately below the dipole, the vertically oriented component of the dipole is coupled to the transverse

¹Department of Physics, King's College London, Strand, London WC2R 2LS, UK. ²Nanophotonics Technology Center, Universitat Politècnica de València, Valencia 46022, Spain.

*Corresponding author. E-mail: a.zayats@kcl.ac.uk (A.V.Z.); frarodfo@ntc.upv.es (F.J.R.-F.)

field of the electromagnetic mode, while the horizontal component of the dipole drives the longitudinal component. Both components have different amplitudes and phases. A properly chosen polarization of the dipole will result in destructive interference in one prescribed direction and a mode propagating in the opposite direction.

Without restriction on the nature of wave-guided modes, we consider a rigorous quantitative description based on phase-matching conditions between the source dipole and the structure's modes. It is convenient to decompose the field of the dipole into spatial-frequency components k_x and k_y (3). The full three-dimensional (3D) treatment is described in (23). Here we discuss a 2D case in which the dipole moment is defined per unit length as $\mathbf{p}_{2D} = [p_x, p_z]$, and the decomposition of its magnetic field can be written as $H_y(x, z) = \int H_y(k_x, z) e^{ik_x x} dk_x$, where

$$H_y(k_x, z) = \frac{i\omega}{8\pi^2} \left[p_z \frac{k_x}{k_z} \mp p_x \right] e^{ik_z |z - z_{\text{dipole}}|} \quad (1)$$

$k_z = (k_0^2 - k_x^2)^{1/2}$ is the wave vector along z , ω is the angular frequency, $k_0 = \omega/c$, and the phasor notation $\mathbf{H}(\mathbf{r}, t) = \text{Re}[\mathbf{H}(\mathbf{r})e^{-i\omega t}]$ was used. The upper sign in Eq. 1 applies to $z > z_{\text{dipole}}$, while the lower sign applies to $z < z_{\text{dipole}}$, where z_{dipole} is the dipole coordinate. The H_y components with $-k_0 < k_x < k_0$ correspond to propagating plane waves forming an angle $\theta = \sin^{-1}(k_x/k_0)$ with the z axis, whereas the components with $|k_x| > k_0$ are evanescent and important only in the near field (for $z \ll \lambda$).

The linear superposition of the two orthogonal dipole orientations p_x and p_z leads to both far-field and near-field interference. The dipole oriented along z axis has an odd parity of its spatial frequency spectrum $H_y(k_x)$, whereas the opposite is true for the x -oriented dipole, which shows an even parity. This suggests that applying the superposition principle to these dipoles could result in a nonsymmetric spatial-frequency distribution. The term (k_x/k_z) in Eq. 1 is real-valued for propagating components and imag-

inary for evanescent ones. This means that to achieve destructive interference [$H_y(k_x) = 0$] inside the light cone ($|k_x| < k_0$), p_x and p_z should be in phase (linearly polarized). Remarkably, the interference can take place outside the light cone ($|k_x| > k_0$) when p_x and p_z are 90° out of phase. According to Eq. 1, for a circularly polarized dipole with $\mathbf{p}_{2D} = p[1, -i]/\sqrt{2}$, the spectral amplitude of the evanescent components with $k_x > 0$ add up destructively, whereas for negative k_x both dipole components reinforce each other (Fig. 1B). This near-field interference effect is the basis for the selective directional excitation of guided modes by the evanescent components of the dipole field. The directional behavior that emerges from the evanescent components is quite a counterintuitive result, considering that no preferred direction is observed for the propagating components of the circular dipole radiation—as can be shown by noting that the magnetic field radiated per unit angle, obtained by substituting $k_x = k_0 \sin \theta$ in Eq. 1, is given by $H_y(\theta, z) d\theta = H_0 e^{\mp i\theta} d\theta$, which has a constant magnitude for all radiating angles θ . From the directional asymmetry of the evanescent component spectrum, it follows that by placing a structure that supports bound propagating modes with an $\exp(\pm ik_x^m x)$ dependence (with $|k_x^m| > k_0$) in the near field of this dipole, the modes propagating toward negative x will be dominantly excited over the modes propagating in the positive x direction with a ratio equal to that of the amplitudes of the corresponding evanescent wave components of the dipole $R = |H_y(k_x^m)/H_y(-k_x^m)|$. The opposite directionality is observed if the direction of rotation of the dipole is reversed. The z position of the dipole does not affect this ratio, because the evanescent decay, $\exp(ik_z |z|)$ in Eq. 1, affects the fields with both signs of k_x equally.

This effect has been demonstrated numerically for two different waveguides by applying the spectral decomposition of the dipole and the transfer matrix method (24). The first example is a dielectric slab of thickness $s = 0.2\lambda$, with permittivity $\epsilon_d = 9 + 0.1i$, placed at a distance $d =$

0.1λ from the dipole. This slab has a bound wave-guided mode that can propagate in opposite directions with the wave vector $\pm k_x^d = \pm 1.965k_0$ (Fig. 1B). Clearly, the mode with $-k_x^d$ will be excited by the circular dipole with a much greater amplitude than the one with $+k_x^d$ (amplitude ratio $R = 13.4$), resulting in unidirectional propagation of the mode within the slab (Fig. 2B). The second example is a semi-infinite metallic medium with permittivity $\epsilon_m = -2 + 0.1i$, placed at a distance $d = 0.3\lambda$ from the circular dipole. The air-metal boundary supports counterpropagating SPP modes with wave vectors $\pm k_x^{\text{spp}} = \pm 1.41k_0$ (Fig. 1B). Only one of these modes is predominantly excited by the circular dipole (amplitude ratio $R = 5.8$), resulting in directional SPP propagation (Fig. 2C). The lobes observed toward the upper right part of the figure correspond to far-field radiation from the dipole and its reflection. Animated versions of Fig. 2, A to D, are given in movies S1 to S4.

In both presented examples, a residual non-zero amplitude is still observed for the modes with positive k_x . This residual wave can be eliminated by using a dipole moment with different relative amplitudes in its x and z components (elliptical polarization). Relying on Eq. 1, it is possible to design a dipole with a spatial frequency spectrum such that the field is exactly zero for the mode that needs to be inhibited; e.g., $H_y(k_x^{\text{spp}}) = 0$ for the wave vector of the mode

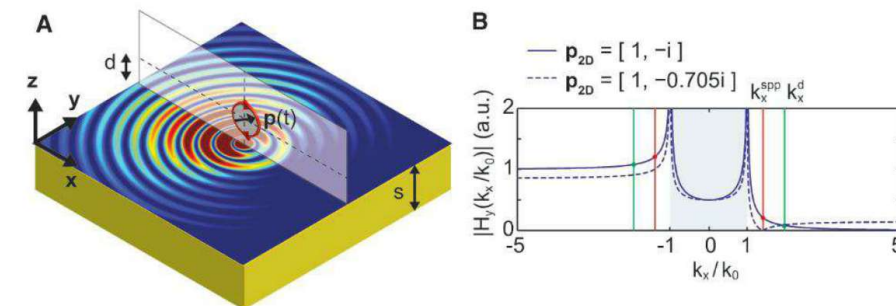


Fig. 1. Asymmetric excitation of waveguided modes. (A) Schematics of the circularly polarized dipole over a planar waveguiding structure (metal-dielectric interface). A cross-section of the calculated magnetic field distribution is overlaid at the surface of a metal ($d = 0.3\lambda$, $\epsilon = -2 + 0.1i$), showing the asymmetric excitation of SPPs. (B) Spatial frequency spectra of a circularly polarized (continuous line) and elliptically polarized (dashed line) dipole.

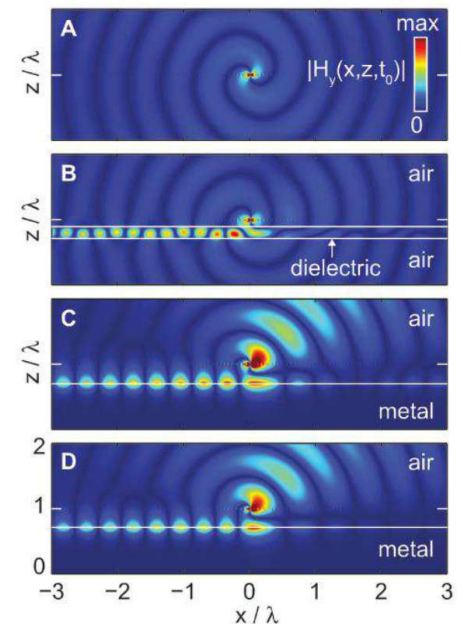
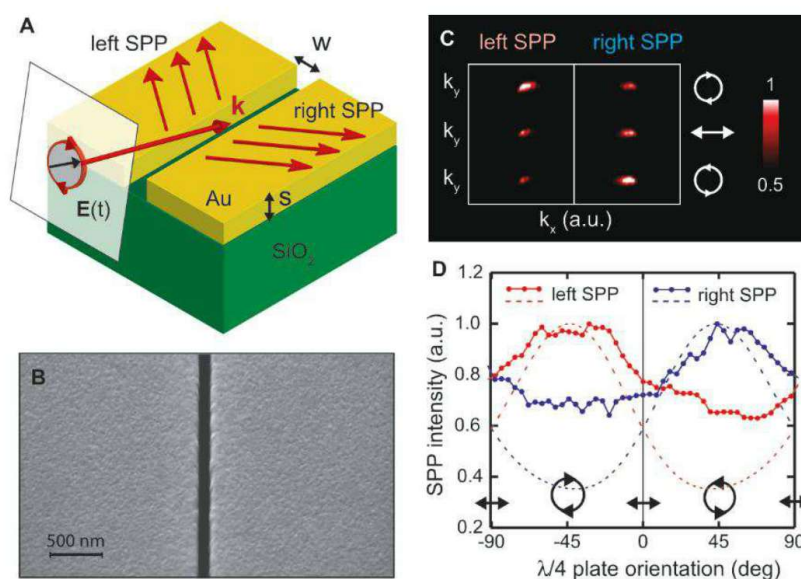


Fig. 2. Dipole radiation in various surroundings. Magnetic field distributions for a 2D circularly polarized dipole $\mathbf{p}_{2D} = [1, -i]$ (A) radiating in free space, (B) exciting a dielectric waveguide mode ($d = 0.1\lambda$, $\epsilon = 9 + 0.1i$, $s = 0.2\lambda$), and (C) exciting SPP mode ($d = 0.3\lambda$, $\epsilon = -2 + 0.1i$, $s = \infty$). (D) Same as (C) but with an optimized elliptical dipole $\mathbf{p}_{2D} = [1, -0.705i]$ to minimize the SPP excitation in the opposite direction. Color scale is the same for all plots.

Fig. 3. Directional SPP excitation with a slit. (A) Schematics of the experiment. A plane wave is incident at nearly grazing angle (70°) onto a slit in a metal film. (B) Scanning electron micrograph of the slit. (C) Spatially filtered Fourier plane images of the SPP leakage radiation collected from the sample for different polarization states of the illuminating light. The bright spots correspond to the points on the SPP wave vector arc for the Au/air interface with k_y matching the incident wave vector. (D) Experimental (solid lines) and simulated (dashed lines) dependences of the intensity of left and right excited SPPs on the polarization of the illuminating light.



with positive k_x^{SPP} . This is the case for $\mathbf{p}_{2D} = [1, -0.705i]$, showing a zero value at exactly the SPP wave vector $k_x^{\text{SPP}} = +1.41k_0$ (Fig. 1B). The resulting truly unidirectional excitation of SPP wave is shown in Fig. 2D, with no coupling to the “undesired” back-propagating SPP mode. The finite lifetime of the SPPs due to the Ohmic losses causing broadening in the wave vector spectrum may, however, result in some minor spurious excitation.

To experimentally demonstrate the concept, we show the directional excitation of SPPs on a smooth 50-nm gold film using a symmetric 130-nm-wide slit illuminated with circularly polarized light. The slit plays the role of the 2D circularly polarized dipole, exciting SPPs on the nearby smooth metal film. Three-dimensional scenarios may be realized by considering single defects in a film (25) or a nanoparticle placed on a metal surface (26). Because the circular polarization of the dipole is required in a plane perpendicular to the metal surface (Fig. 1A), the illumination is chosen at a grazing angle along the slit length (Fig. 3A). The leakage radiation of the SPPs into the glass substrate is recorded as bright spots on a charge-coupled device camera placed in a spatially filtered Fourier plane (fig. S1). In the case of linear polarization, both SPPs are excited equally on both sides of the slit, whereas for left- and right-handed circular polarizations, SPPs are predominantly excited propagating to the right and left side of the slit, respectively (Fig. 3C).

A full set of polarization states was studied by rotating a $\lambda/4$ plate in a linearly polarized excitation beam (Fig. 3D). For linear polarization ($\lambda/4$ plate orientation: 0° , -90° and $+90^\circ$), the expected behavior of equal excitation of SPPs on both sides of the slit is observed. However, for two circular polarizations of the incident light, SPPs are preferentially excited in one direction. Symmetry requires that the two SPP excitation

curves be mirror images of each other, which is clearly observed in the experiment. Small deviations from theoretical predictions obtained for an ideal dipole can be expected to originate from the finite size and shape of the slit, whose dipolar behavior is not equivalent for fields polarized across the slit and perpendicularly to it. This prevents achieving full directionality and contributes to the observed background intensity (Fig. 3D). A complete control of the polarization ellipse of incoming light (I_0 , I_1) would optimize the device performance, which, following symmetry considerations (23), could potentially achieve a 1:0 contrast ratio. Similarly, directional SPP excitation can be achieved with a slit grating by using the same approach (fig. S2).

We have described a fundamental example of vectorial near-field interference using a simple circularly or elliptically polarized dipole to achieve directional broadband excitation of guided modes. The underlying physics holds potential and wide applicability to other optical processes. Useful applications may be in the excitation of photonic guided modes exhibiting directional dependence with the direction of the input circularly polarized light, enabling compact nanoscale light sources that could efficiently absorb incoming illumination and couple it to the modes of Si-photonics or plasmonic guides in a desired single direction. It also opens exciting possibilities for directional switching, polarization sorting, and processing of polarization-encoded information, including polarization-entangled optical qubits.

References and Notes

1. N. Yu *et al.*, *Science* **334**, 333 (2011).
2. X. Ni, N. K. Emani, A. V. Kildishev, A. Boltasseva, V. M. Shalae, *Science* **335**, 427 (2012).
3. L. Novotny, B. Hecht, *Principles of Nano-Optics* (Cambridge University Press, 2011).
4. R. Merlin, *Science* **317**, 927 (2007).

5. J. B. Pendry, *Phys. Rev. Lett.* **85**, 3966 (2000).
6. L. E. Helseth, *Opt. Commun.* **281**, 1981 (2008).
7. G. V. Eleftheriades, A. M. H. Wong, *IEEE Microw. Wirel. Co.* **18**, 236 (2008).
8. J. Y. Lee *et al.*, *Nature* **460**, 498 (2009).
9. M. I. Stockman, S. V. Faleev, D. J. Bergman, *Phys. Rev. Lett.* **88**, 067402 (2002).
10. M. Aeschlimann *et al.*, *New J. Phys.* **14**, 033030 (2012).
11. M. Sukharev, T. Seideman, *Nano Lett.* **6**, 715 (2006).
12. W. L. Barnes, A. Dereux, T. W. Ebbesen, *Nature* **424**, 824 (2003).
13. J. A. Schuller *et al.*, *Nat. Mater.* **9**, 193 (2010).
14. H. Kim, B. Lee, *Plasmonics* **4**, 153 (2009).
15. N. Bonod, E. Popov, L. Li, B. Chernov, *Opt. Express* **15**, 11427 (2007).
16. J.-S. Bouillard, S. Vilain, W. Dickson, G. A. Wurtz, A. V. Zayats, *Scientific Reports* **2**, 829 (2012).
17. X. Li, Q. Tan, B. Bai, G. Jin, *Appl. Phys. Lett.* **98**, 251109 (2011).
18. I. P. Radko *et al.*, *Opt. Express* **17**, 7228 (2009).
19. Y. Liu *et al.*, *Nano Lett.* **12**, 4853 (2012).
20. A. G. Curto *et al.*, *Science* **329**, 930 (2010).
21. M. Laroche *et al.*, *Opt. Lett.* **30**, 2623 (2005).
22. G. Roelkens *et al.*, *J. Nanosci. Nanotechnol.* **10**, 1551 (2010).
23. Supplementary materials are available on Science Online.
24. W. N. Hansen, *J. Opt. Soc. Am.* **58**, 380 (1968).
25. N. Rotenberg *et al.*, *Phys. Rev. Lett.* **108**, 127402 (2012).
26. Z. Ruan, S. Fan, *Phys. Rev. Lett.* **105**, 013901 (2010).

Acknowledgments: This work has been supported in part by the Engineering and Physical Sciences Research Council (grant EP/H000917/2). F.J.R.-F. acknowledges support from grant FPI of Generalitat Valenciana. A.M. acknowledges financial support from the Spanish government (contracts Consolider EMET CSD2008-00066 and TEC2011-28664-C02-02). P.G. acknowledges the Royal Society for a Newton International Fellowship.

Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6130/328/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S3
Movies S1 to S4

6 December 2012; accepted 12 March 2013
10.1126/science.1233739

Polarization-Controlled Tunable Directional Coupling of Surface Plasmon Polaritons

Jiao Lin,^{1,2*} J. P. Balthasar Mueller,^{1*} Qian Wang,³ Guanghui Yuan,³ Nicholas Antoniou,⁴ Xiao-Cong Yuan,⁵ Federico Capasso^{1†}

Light can be coupled into propagating electromagnetic surface waves at a metal-dielectric interface known as surface plasmon polaritons (SPPs). This process has traditionally faced challenges in the polarization sensitivity of the coupling efficiency and in controlling the directionality of the SPPs. We designed and demonstrated plasmonic couplers that overcome these limits using polarization-sensitive apertures in a gold film. Our devices enable polarization-controlled tunable directional coupling with polarization-invariant total conversion efficiency and preserve the incident polarization information. Both bidirectional and unidirectional launching of SPPs are demonstrated. The design is further applied to circular structures that create radially convergent and divergent SPPs, illustrating that this concept can be extended to a broad range of applications.

Surface plasmon polaritons (SPPs) are propagating excitations that arise from the coupling of light with collective oscillations of the electrons at the surface of a metal (*1*). The strong dependence of their properties on the composition and structure of the surface has led to many advances in the control of light at the nanoscale (2–9), holding promise for optical information technology and optoelectronics (10, 11) and for the study and engineering of light-matter interaction (12, 13).

The polarization states of the optical signal that can be coupled to a plasmonic device are often limited by the selectivity of the coupling process. For directional SPP excitation, usually only the component of the incident light that is polarized perpendicularly to either groove- or ridge-like scattering elements (in the case of gratings) or to the metal surface itself (in the case of prism-based schemes) can be coupled into SPPs (*1*). Light in the orthogonal polarization does not couple to SPPs, leading to a decrease in the SPP signal and a loss of information about the incident polarization state.

Additional challenges arise in controlling the direction of propagation of the generated SPPs. Poor directionality frequently represents a substantial source of noise and reduces efficiency. This has led to the development of several schemes that couple unidirectionally, albeit with limited dynamic tunability (14–16).

We present a directional plasmonic coupler that addresses these challenges by offering

polarization-invariant coupling efficiency to SPPs while allowing full control over the distribution of power between two counter-propagating SPP modes. This includes unidirectional coupling and preserves polarization information.

A narrow aperture in a metal film selectively scatters incident light that is polarized perpendicularly to it, giving rise to SPPs. This occurs both for light incident on the front and for light incident on the back of apertures that fully perforate the film. In the latter case, SPPs are transmitted through the apertures to the other side of the film, where they are launched across the surface. The SPP emission pattern of a subwavelength aperture is approximately that of an in-plane dipole (17), which is known analytically (18) and plotted in Fig. 1A along with the corresponding orientation of the aperture. When many such dipoles are arranged in a column with a spacing that is smaller than the SPP wavelength (λ_{SPP}), the launched SPPs are plane waves that propagate perpendicularly away toward either side of the column (Fig. 1B). This effect is independent of the orientation of the dipoles of the column. We can therefore create a source of SPP plane waves propagating in a fixed direction that can be designed to selectively respond to a polarization component of the incident light determined by the orientation of the apertures of the column.

A more complex polarization response can be achieved by fabricating multiple columns on the metal surface, resulting in interference of the SPP waves created by individual columns. We consider two parallel columns (Fig. 1C) that are spaced a distance S apart and are made up of apertures with width W and length L , such that $W \ll L < \lambda_{\text{SPP}}$. The apertures of one column are spaced a distance D apart, with $D < \lambda_{\text{SPP}}$. The columns are offset by half a period ($D/2$) along their axes to reduce near-field coupling and scattering of the SPPs by neighboring apertures. With this geometry, simulations showed both effects to be negligible (19). The apertures of the first and second column are oriented at angles

θ_1 and θ_2 with respect to the y axis. If light with an electric field $\vec{E}$ is normally incident on the metal surface, the respective columns will then couple to the components of the incident field $\vec{E}_1$ and $\vec{E}_2$ that are polarized at angles $90^\circ + \theta_1$ and $90^\circ + \theta_2$. The conversion efficiency C_i of a column scales as $C_i \propto \cos^2 \theta_i$ because the SPP emission pattern of the individual apertures is anisotropic (Fig. 1A) and only the component of the wave that is launched perpendicular to the column is not cancelled by the fields of the surrounding dipoles (Fig. 1B).

The field components $\vec{E}_1$ and $\vec{E}_2$ can have a relative phase δ that is preserved by the coupling process and transferred to the SPPs. If the propagation loss of the SPPs over the small distance S is neglected, interference of the SPP waves launched by each column results in time-averaged SPP field intensities propagating to the right (I_R) and the left (I_L) to the pair of columns (19):

$$\begin{aligned} I_R &\propto [(C_1 E_1^2 + C_2 E_2^2) + 2E_1 E_2 \cos(k_{\text{SPP}} S + \delta)] \\ I_L &\propto [(C_1 E_1^2 + C_2 E_2^2) + 2E_1 E_2 \cos(k_{\text{SPP}} S - \delta)] \end{aligned} \quad (1)$$

where the intensities are calculated in the dielectric. E_1 and E_2 denote the amplitudes of the field components coupled to the respective columns and $k_{\text{SPP}} = \frac{2\pi}{\lambda_{\text{SPP}}}$ the SPP wave number. If $|\theta_1 - \theta_2| = 90^\circ$, then $|\vec{E}|^2 = |\vec{E}_1|^2 + |\vec{E}_2|^2$. We make the particular choice of $\theta_1 = 45^\circ$ and $\theta_2 = 45^\circ + 90^\circ = 135^\circ$, in which case the coupling coefficients of the two columns $C_1 = C_2 = C$. If we adjust the lateral spacing S of the columns with respect to the SPP wavelength to $S = \frac{\pi}{2} k_{\text{SPP}}^{-1}$, Eq. 1 becomes

$$\begin{aligned} I_R &\propto C[(E_1^2 + E_2^2) - 2E_1 E_2 \sin\delta] \\ I_L &\propto C[(E_1^2 + E_2^2) + 2E_1 E_2 \sin\delta] \end{aligned} \quad (2)$$

Notably, the combined intensity of the coupled SPP fields $I_R + I_L \propto C|\vec{E}|^2$ is proportional to the intensity of the incident light but is independent of δ and of the relative magnitude of the coupled field components E_1 and E_2 . The total power that is converted to SPPs can thus be made independent of the polarization of the incident light. Moreover, the polarization dependence in Eq. 2 can be used to adjust the fraction of the total converted power that is propagating in the SPP waves launched either toward the left or toward the right side of the column pair by changing the polarization state of the incident light. The distribution of the intensities in the counter-propagating SPP modes as a function of polarization as predicted by Eq. 2 is shown in Fig. 2A. Here, we expressed the polarization state of the incident light in complex number representation (20) in terms of ψ and δ according to

$$e^{-i\delta} \tan \psi = \frac{E_2^c}{E_1^c} \quad (3)$$

where E_1^c and E_2^c are the complex amplitudes of the field components $\vec{E}_1$ and $\vec{E}_2$, respectively.

¹School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138, USA. ²Singapore Institute of Manufacturing Technology, 71 Nanyang Drive, Singapore 638075, Singapore. ³School of Electrical and Electronic Engineering, Nanyang Technological University, Nanyang Avenue, Singapore 639798, Singapore. ⁴Center for Nanoscale Systems, Harvard University, Cambridge, MA 02138, USA. ⁵Institute of Modern Optics, Key Laboratory of Optical Information Science and Technology, Ministry of Education of China, Nankai University, Tianjin 300071, China.

*These authors contributed equally to this work.

†Corresponding author. E-mail: capasso@seas.harvard.edu

Unidirectional coupling occurs when the incident light is circularly polarized ($E_1 = E_2; \frac{\pi}{2}$), with the direction determined by the handedness. In this

way, the two circular polarization states represent two separate channels that can selectively address structures to either side of the coupler. Switchable

unidirectional coupling is possible by changing the handedness of the incident light (Fig. 2A). The structure is analogous to a polarizing beam

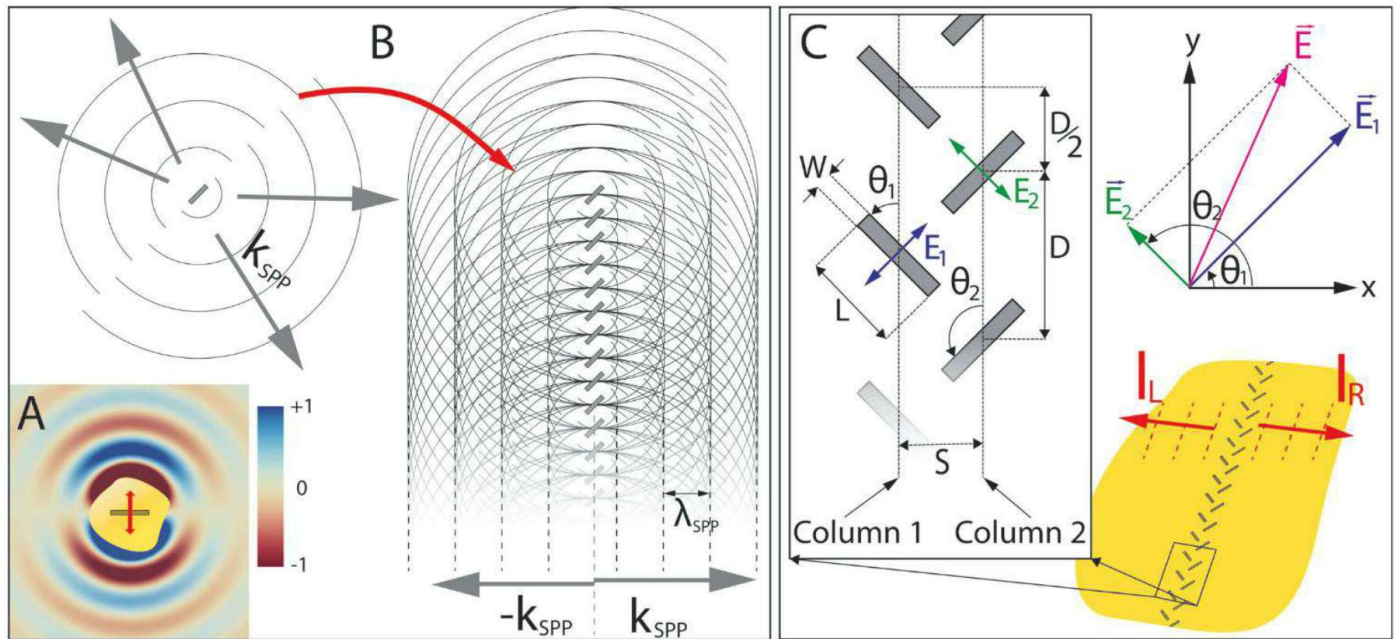
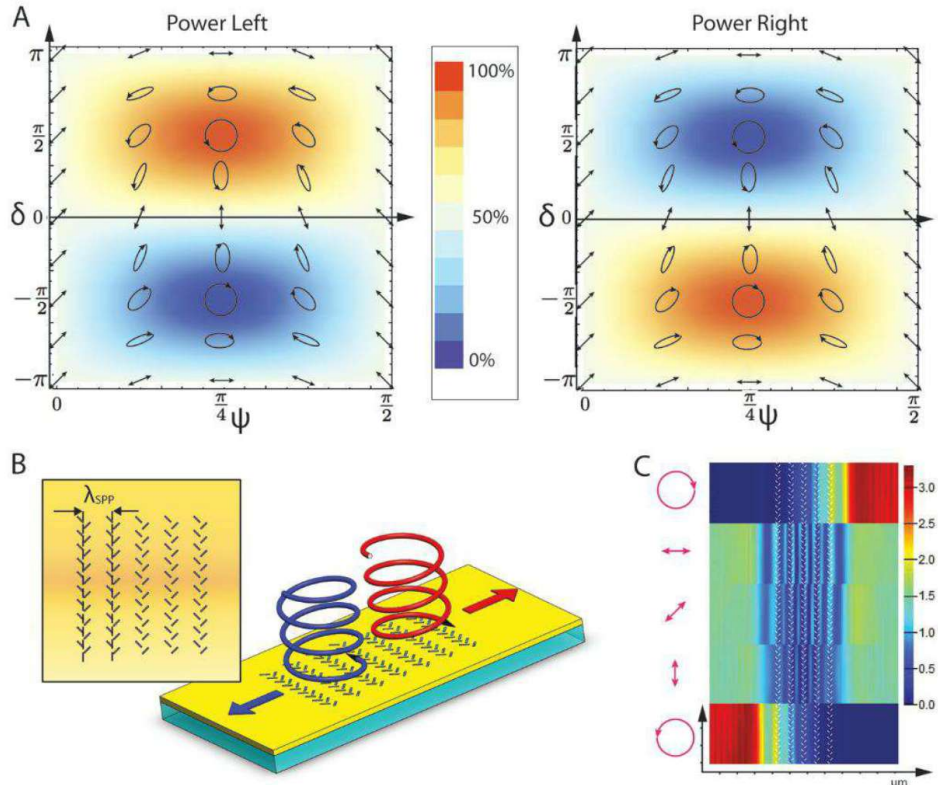


Fig. 1. Closely spaced subwavelength apertures as polarization-selective SPP plane-wave sources. (A) The calculated normal component of the SPP electric field launched by an in-plane dipole (in arbitrary units), which is an approximation for a subwavelength aperture in a gold film (overlay) that is scattering incident light polarized perpendicular to its long axis (polarization indicated by a red arrow). (B) The SPP waves generated by a single aperture propagate radially away from it, with wavefronts shown in black. The waves

emitted from many apertures arranged in a column with subwavelength spacing interfere constructively along planes parallel to the column, shown as dashed lines. SPP wave vectors (k_{SPP}) are shown as gray arrows. (C) Two columns of apertures (1 and 2) are positioned in parallel with spacing S . The columns couple to the field components $\vec{E}_1$ (blue arrows) and $\vec{E}_2$ (green arrows) of the incident field $\vec{E}$ (pink arrow). The resulting combined SPP plane waves propagating away from the column pair (red arrows) have intensities I_R and I_L .

Fig. 2. Changing the incident polarization enables continuous tuning of the directionality of the launched SPPs. (A) Distribution of power between the SPP waves propagating to either side of the coupler as a function of the polarization state of the normally incident light according to Eq. 2. The incident polarization states are expressed in complex number notation in terms of the angles Ψ and δ (Eq. 3) and additionally shown as a black overlay. (B) Multiple parallel column pairs are spaced λ_{SPP} apart. The structure is shown such that right (left) CPL is coupled to the right (left) when it is incident from the top. (C) FDTD simulation of the near-field intensity (arbitrary units) above a coupler made up of five column pairs (white overlay) under illumination by incident light with linear and circular polarizations (pink arrows).



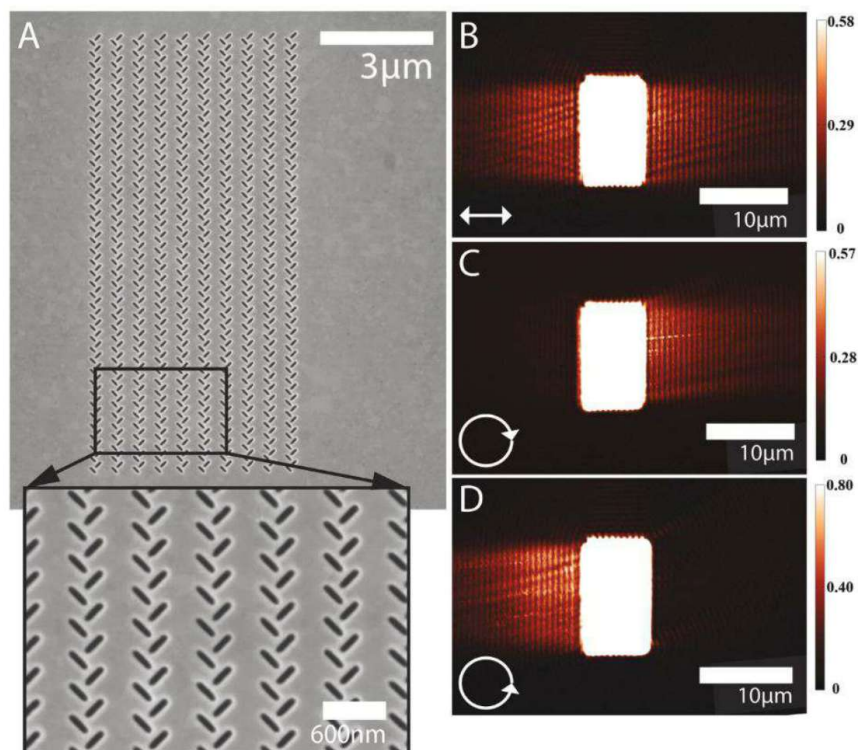


Fig. 3. Measurement of the SPPs launched by a fabricated coupler. (A) Scanning electron microscope (SEM) image of a structure fabricated in a gold film for operation at $\lambda = 633$ nm. (B to D) NSOM images of the structure under illumination from the back by LPL (B), right CPL (C), and left CPL (D). The state of incident polarization is shown by white arrows.

splitter with two SPP output channels because the SPP fields propagating to the left and the right correspond to the circularly polarized components of the incident light. The polarization state of the incident light is then fully encoded in the amplitude and relative phase of the SPPs.

The cross-section of the coupler can be increased by placing several column pairs an SPP wavelength apart in the manner of a second-order grating (Fig. 2B). The side of the structure toward which each circularly polarized state is routed relative to the observer is shown in Fig. 2B.

We performed full-wave finite-difference time-domain (FDTD) simulations of the SPPs created by a group of five column pairs patterned on a gold surface for various incident polarizations. This structure was designed for operation at $\lambda = 633$ nm, corresponding to $\lambda_{\text{SPP}} \approx 606$ nm. The geometrical parameters of the structure are $S = 150$ nm, $D = 300$ nm, $W = 40$ nm, and $L = 200$ nm. The column pairs are spaced 600 nm apart, and the thickness of the Au film is 200 nm. The results show good agreement with the predictions of Eq. 2 (Fig. 2C). Further simulation results can be found in (19).

We fabricated 10 column pairs, with $S = 150$ nm, $D = 300$ nm, $W = 50$ nm, and $L = 200$ nm in a 150-nm-thick gold film using focused ion beam milling (FIB). The devices were back-illuminated with polarized 633-nm laser light, and the SPPs were measured using near-field

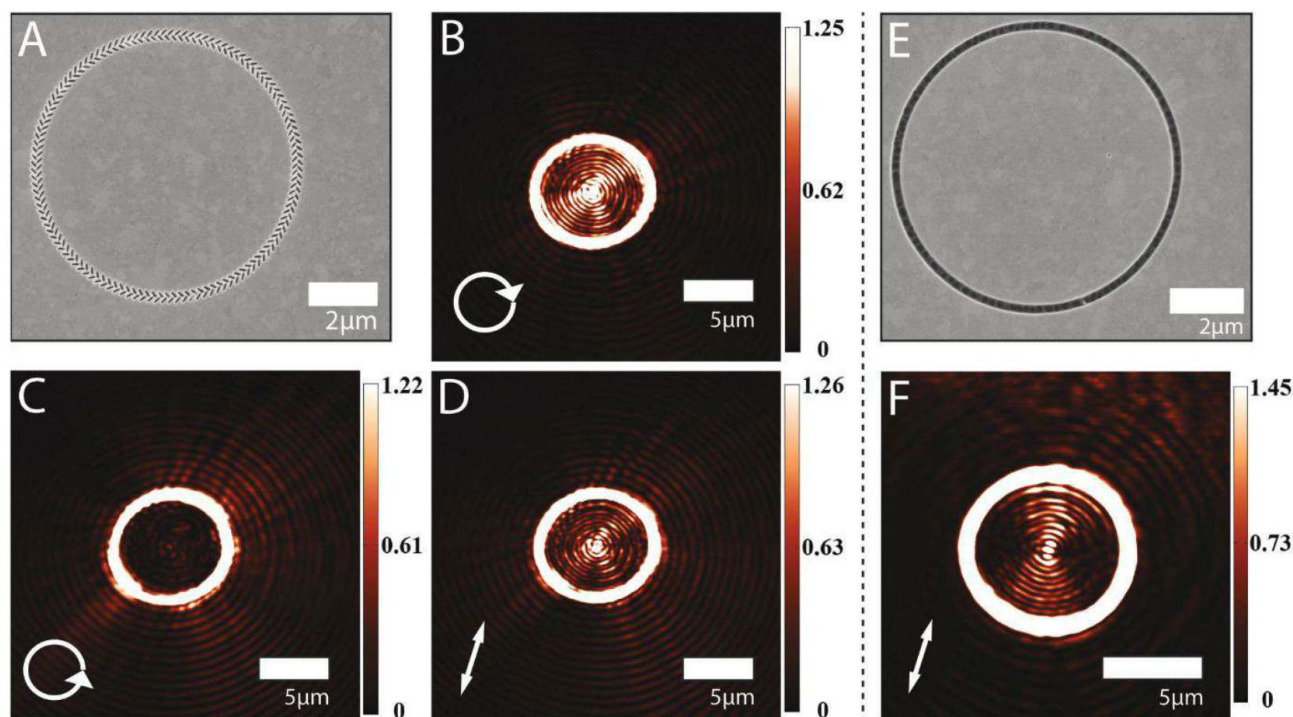


Fig. 4. Curved structures and polarization-invariant coupling efficiency. (A) SEM image of a circular coupler based on our design. (B) NSOM images of the coupler under right CPL, resulting in radially inward coupling, which creates a high-intensity standing wave within the circle. (C) NSOM image under left CPL, resulting in radial unidirectional coupling away from the circle and low intensity within the circle. (D) NSOM image of the coupler under

LPL, which results in equal power propagating inward and outward. SPPs are generated by sections of the coupler oriented at all angles with respect to the incident polarization. (E) SEM image of a circular groove of the same radius, representative of a conventional plasmonic coupler. (F) NSOM image of the circular groove under LPL, showing generation of SPPs only where the groove is perpendicular to the incident polarization.

scanning optical microscopy (NSOM) (19). The observed field distributions are in good agreement with the theoretical model (Fig. 3). The NSOM micrographs exhibit fringe patterns that result from the interference of the SPPs with incident light directly transmitted through the film, confirming that the measured optical signal corresponds to the near-field (19). Figure 3, B to D, shows in particular that circularly polarized light (CPL) of opposite handedness generates counter-propagating SPP beams, whereas linearly polarized light (LPL) launches SPP beams of equal intensity toward either side of the coupler.

This coupling scheme can be generalized by adding curvature to the column pairs on the metal surface. A coupler bent into a circle can be used to achieve radial unidirectional coupling (Fig. 4A). Depending on the handedness of the structure, the two circular polarization states result in SPPs that propagate radially toward the center of the circle (Fig. 4B) or outward (Fig. 4C). In case of unidirectional inward coupling, a high-intensity standing wave is formed within the circle, whereas the field intensity is minimal in the case of unidirectional outward coupling. LPL, as an equal superposition of left and right circularly polarized waves, results in SPPs propagating equally inward and outward (Fig. 4D). SPPs emanate from sections of the coupler oriented at all angles with respect to the incident polarization, demonstrating the polarization-invariant coupling efficiency for LPL. This feature becomes clear by comparing the properties of our structure with those of a ring-shaped groove (Fig. 4E), which is functionally equivalent to conventional SPP

couplers. The NSOM image of the SPP field above the latter shows that SPPs can only be generated where the incident polarization is perpendicular to the groove (Fig. 4F).

Our couplers may form the basis for novel plasmonic devices and can be used to both improve efficiency and lower cross-talk in plasmonic networks. The possibility of encoding the polarization information in SPPs is particularly relevant based on recent evidence that SPPs preserve quantum coherence (21), which suggests that the structure may be used to reversibly convert polarization-encoded photon qubits to SPPs. This could help to further harness the unique properties of SPPs for applications in quantum information.

References and Notes

1. S. A. Maier, *Plasmonics: Fundamentals and Applications* (Springer-Verlag, New York, 2007).
2. M. L. Juan, M. Righini, R. Quidant, *Nat. Photonics* **5**, 349 (2011).
3. S. Nie, S. R. Emory, *Science* **275**, 1102 (1997).
4. S. Kim *et al.*, *Nature* **453**, 757 (2008).
5. P. Genevet *et al.*, *Appl. Opt.* **50**, G56 (2011).
6. H. J. Lezec, J. A. Dionne, H. A. Atwater, *Science* **316**, 430 (2007).
7. T. Kosako, Y. Kadoya, H. F. Hofmann, *Nat. Photonics* **4**, 312 (2010).
8. N. Yu *et al.*, *Science* **334**, 333 (2011).
9. N. Yu, Q. Wang, F. Capasso, *Laser Photonics Rev.* **6**, 24 (2012).
10. E. Ozbay, *Science* **311**, 189 (2006).
11. M. L. Brongersma, V. M. Shalae, *Science* **328**, 440 (2010).
12. H. A. Atwater, A. Polman, *Nat. Mater.* **9**, 205 (2010).
13. A. G. Curto *et al.*, *Science* **329**, 930 (2010).
14. F. López-Tejeda *et al.*, *Nat. Phys.* **3**, 324 (2007).
15. A. Baron *et al.*, *Nano Lett.* **11**, 4207 (2011).
16. Y. Liu *et al.*, *Nano Lett.* **12**, 4853 (2012).
17. T. Tanemura *et al.*, *Nano Lett.* **11**, 2693 (2011).
18. A. Archambault, T. V. Teperik, F. Marquier, J. J. Greffet, *Phys. Rev. B* **79**, 195414 (2009).
19. See supplementary materials on Science Online.
20. A. Yariv, P. Yeh, *Photonics: Optical Electronics in Modern Communications*; Oxford Series in Electrical and Computer Engineering (Oxford Univ. Press, New York, 2006).
21. G. Di Martino *et al.*, *Nano Lett.* **12**, 2504 (2012).

Acknowledgments: Part of this work was performed at the Center for Nanoscale Systems (CNS), a member of the National Nanotechnology Infrastructure Network (NNIN), which is supported by the NSF (under award ECS-0335765). CNS is part of Harvard University. This research is supported in part by the Air Force Office of Scientific Research under grant FA9550-12-1-0289. J.L. acknowledges the fellowship support from the Agency for Science, Technology and Research (A*STAR), Singapore. X.-C.Y. acknowledges the support given by the National Natural Science Foundation of China under grants 61036013 and 61138003, the Ministry of Science and Technology of China under grant 2009DFA52300 for China-Singapore collaborations, and the National Research Foundation of Singapore under grant NRF-G-CRP 2007-01. The authors express thanks to M. Kats (School of Engineering and Applied Sciences, Harvard University), K. A. C. Lee and Y. Zhou (Singapore Institute of Manufacturing Technology), and L. Du (Nanyang Technological University) for their assistance in the preparation of the manuscript. Data are available upon request.

Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6130/331/DC1

Materials and Methods

Figs. S1 to S7

Table S1

References (22, 23)

6 December 2012; accepted 27 February 2013

10.1126/science.1233746

External Quantum Efficiency Above 100% in a Singlet-Exciton-Fission–Based Organic Photovoltaic Cell

Daniel N. Congreve,* Jiye Lee,* Nicholas J. Thompson,* Eric Hontz, Shane R. Yost, Philip D. Reusswig, Matthias E. Bahlke, Sebastian Reineke, Troy Van Voorhis, Marc A. Baldo†

Singlet exciton fission transforms a molecular singlet excited state into two triplet states, each with half the energy of the original singlet. In solar cells, it could potentially double the photocurrent from high-energy photons. We demonstrate organic solar cells that exploit singlet exciton fission in pentacene to generate more than one electron per incident photon in a portion of the visible spectrum. Using a fullerene acceptor, a poly(3-hexylthiophene) exciton confinement layer, and a conventional optical trapping scheme, we show a peak external quantum efficiency of $(109 \pm 1)\%$ at wavelength $\lambda = 670$ nanometers for a 15-nanometer-thick pentacene film. The corresponding internal quantum efficiency is $(160 \pm 10)\%$. Analysis of the magnetic field effect on photocurrent suggests that the triplet yield approaches 200% for pentacene films thicker than 5 nanometers.

Conventional solar cells generate one electron for each photon that is absorbed. The output voltage is defined by the bandgap, and solar cells waste any excess photon energy as heat. Summing the thermal loss over the solar spectrum yields the Shockley-Queisser efficiency

limit of 34% for solar cells containing a single, optimized semiconductor junction (1).

Splitting excited states, or excitons, generated after the absorption of high-energy photons presents one pathway beyond the single junction efficiency limit. Instead of harvesting a single

electron, several charges can be obtained by dissociating the child excitons. For example, so-called multiple exciton generation mechanisms have been used to produce an average of more than one electron from an ultraviolet photon with energy four times the bandgap (2).

Singlet exciton fission is a type of multiple exciton generation mechanism found in organic semiconductors (3, 4). It is notable because spin conservation disallows the usual competing loss process: thermal relaxation of the high-energy exciton into a single low-energy exciton. In fission, the low-energy exciton is a dark state, inaccessible by a direct transition from either the high-energy exciton or the ground state. Only the evolution of the high-energy state into two dark excitons is spin-allowed. Consequently, prior studies have suggested that singlet fission can be efficient even in the visible spectrum, harnessing photons of just twice the energy of the child excitons (5–10).

There is a side effect of spin in singlet fission, however. The dark exciton controls the electrical properties of the cell. These are decoupled from

Energy Frontier Research Center for Excitonics, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

*These authors contributed equally to this work.

†Corresponding author. E-mail: baldo@mit.edu

the optical absorption, which is controlled by the bright, high-energy exciton. Thus, fission does not itself increase the power efficiency of a solar cell. It potentially doubles the photocurrent at the cost of losing at least half the open circuit voltage. To overcome the Shockley-Queisser lim-

it, solar cells could combine fission with a conventional material that fills in the absorption spectrum above the dark exciton (11–15). First, however, singlet fission must demonstrate that it can break the conventional barrier of one electron per photon.

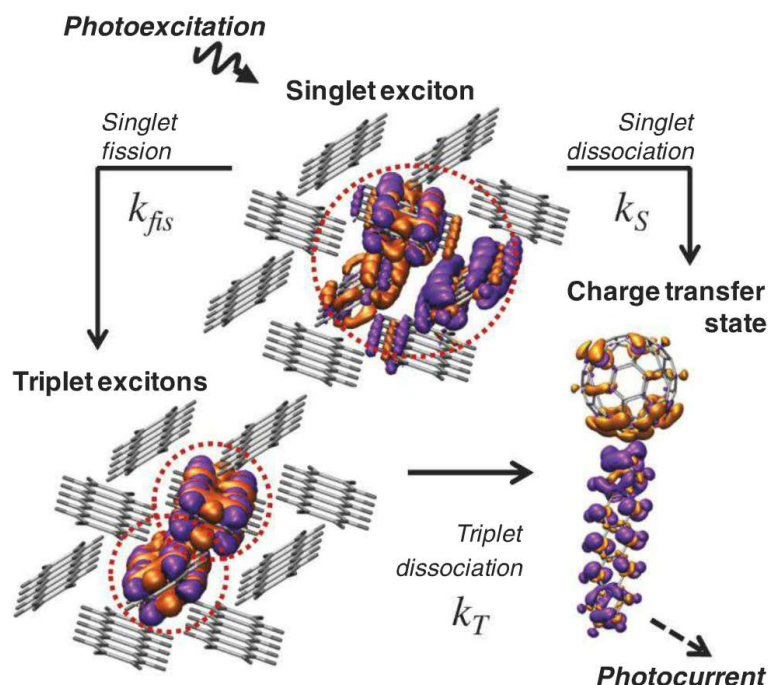


Fig. 1. Singlet fission dynamics in pentacene. Calculations of singlet and triplet excitons and charge transfer states at the pentacene/fullerene interface are shown, with the purple (orange) density indicating where less (more) electron density is found in the excited state. The delocalized singlet exciton and two localized triplet excitons are circled in red. The loss pathway for singlet excitons is direct dissociation into charge before singlet exciton fission.

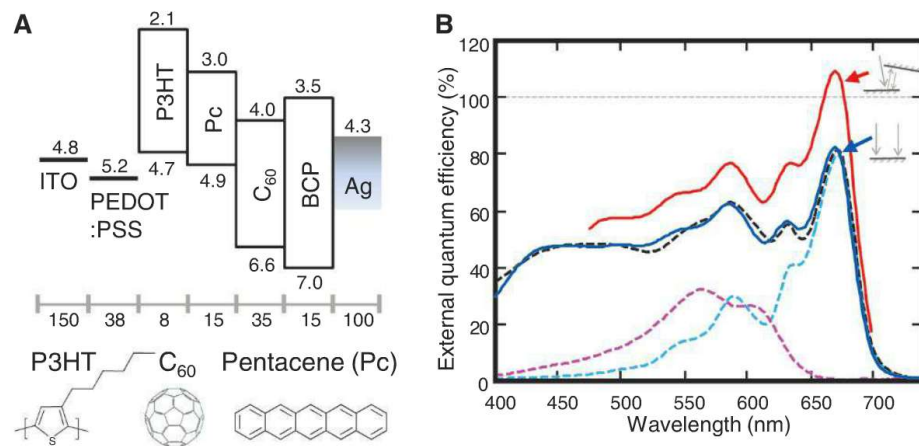


Fig. 2. Device architecture and EQE of a pentacene solar cell. (A) Chemical structures and architecture of the solar cell with the thickness of each layer in nanometers and energy levels of the lowest unoccupied and highest occupied molecular orbitals in electron volts (12, 18, 20, 29–31). The anode is composed of indium tin oxide (ITO) and poly(3,4-ethylenedioxythiophene) poly(styrenesulfonate) (PEDOT:PSS). The cathode employs bathocuproine (BCP) and a silver cap. (B) External quantum efficiency of devices without optical trapping (blue line), and device measured with light incident at 10° from normal with an external mirror reflecting the residual pump light (red line). Optical fits from IQE modeling are shown with dashed lines: modeled pentacene EQE (blue dashes), modeled P3HT EQE (purple dashes), and modeled device EQE (black dashes) for comparison to the measured device efficiency without optical trapping.

The best understood fission material to date is pentacene, an acene with five rings. Its dynamics are illustrated in Fig. 1. Optical excitation generates a delocalized spin 0, or singlet, exciton. Within about 80 fs (5–10), the pentacene singlet exciton splits into a pair of spin 1, or triplet, excitons. A pair of triplet excitons can be combined in nine different spin states. As some triplet-pair states have singlet character (total spin of 0), singlet fission is spin-allowed. Under an applied magnetic field, the singlet character of the triplet-pair states is redistributed, changing the number of states with singlet character. Thus, the rate of singlet fission is dependent on the local magnetic field, offering a unique probe of fission dynamics in thin films and devices (4, 11, 12, 16, 17). Although triplet excitons are dark states, energy may be extracted from them if they are dissociated into charge. This is possible at a junction between pentacene and the fullerene C_{60} when the pentacene is oriented approximately perpendicular to the interface (12).

The structure of our pentacene-based solar cell is shown in Fig. 2A. The core of the device is a pentacene/ C_{60} donor-acceptor junction (4, 12, 18). To minimize triplet exciton losses, we used a thin pentacene layer and also introduced an exciton blocking layer of regio-regular poly(3-hexylthiophene) (P3HT), which was placed between the pentacene and the anode. The combination of the wide energy gap and 1.5-eV triplet energy (19) of P3HT confines pentacene triplet excitons, and its highest occupied molecular orbital (HOMO) of 4.7 eV (20) helps extract holes from pentacene. To maximize light absorption, devices were fabricated with MgF_2 antireflection coatings on the front surface of the glass substrate (21).

The external quantum efficiency (EQE) is defined as the ratio between the number of electrons flowing out of the device and the number of photons incident upon it. We measured the EQE (Fig. 2B) for a device that features a 15-nm-thick pentacene layer. The EQE at normal incidence is $(82 \pm 1)\%$ at the peak pentacene absorption wavelength $\lambda = 670$ nm. Optical modeling (21) predicts that the internal quantum efficiency (IQE), which is defined as the number of electrons collected per photon absorbed, for photoexcitation of pentacene and P3HT is $(160 \pm 10)\%$ and $(150 \pm 10)\%$, respectively. The IQE of pentacene in this structure is approximately double that reported previously for pentacene (18, 22), and the high IQE of P3HT is consistent with the expected sensitization of P3HT by pentacene, as singlet excitons generated in P3HT are transferred to pentacene and then split into triplets (23). The peak EQE drops to 24% when P3HT is absent. The P3HT appears to block triplet diffusion to the anode and suppress recombination by improving hole extraction; see figs. S1 to S3 and accompanying text for further discussion of both sensitization and the efficiency enhancement due to P3HT.

The 15-nm-thick film of pentacene in the solar cell microcavity absorbs only 49% of the incident light at $\lambda = 670$ nm according to optical

modeling; hence, the efficiency should improve if a light-trapping scheme is employed. Therefore, we measured the EQE in configurations designed to simulate two conventional optical trapping schemes. The first scheme mounts the cell at 45° to the incident light, with a mirror that directs reflected photons back to the device. This configuration models a sawtooth geometry such that incident light bounces at least twice within the structure (24, 25). In the second scheme, the incident angle is reduced to 10° from the normal, modeling an optical collector that focuses light through a small hole in a mirror held parallel to the surface of the cell (26). The peak EQE at $\lambda = 670$ nm for the solar cell mounted at 45° is $(102 \pm 1)\%$, increasing to $(109 \pm 1)\%$ for incidence at 10° from the normal. Both light-trapping schemes yield efficiencies that meet or exceed the one electron per incident photon benchmark.

The current-voltage characteristics of the planar pentacene solar cell are shown in Fig. 3. The short-circuit current measured at AM1.5 matches the integrated EQE measured at <1 mW/cm 2 to within 6%, demonstrating that the fission process in pentacene is not significantly intensity dependent. As expected, the enhanced EQE does not correspond to a high power efficiency. The open circuit voltage is 0.36 V, identical to the values of previous pentacene devices (18). It is defined by the pentacene triplet energy of 0.86 eV (3, 14). With C $_{60}$ as the acceptor, the device absorbs light only above the pentacene singlet energy at 1.8 eV. Consequently, the power efficiency is $(1.8 \pm 0.1)\%$.

Independent confirmation of the high internal quantum efficiency within the cell is provided by analysis of the photocurrent under a magnetic field. The crucial rates are identified in Fig. 1. The singlet exciton can either directly dissociate into a single electron-hole pair, k_S , or undergo fission resulting in the generation of two electron-hole pairs, $k_{fis}(B)$. In absence of a magnetic field, three out of nine triplet-pair states have singlet character. Under a high magnetic field ($B > 0.2$ T), the number of triplet-triplet pairs with singlet character reduces to two, reducing the singlet fission rate, $k_{fis}(B)$. The photocurrent yield changes

if there is effective competition between fission and the dissociation of the singlet exciton. It is not possible to generate a magnetic field effect on the photocurrent yield unless there is a singlet loss mechanism that competes with the fission process.

For convenience, we write $k_{fis}(B) = \chi(B)k_{fis}^0$, where $\chi(B)$ is the modulation of the zero-field fission rate k_{fis}^0 . The normalized change in photocurrent in steady state, δ , is then given by Eq. 1:

$$\delta = \frac{I(B) - I(0)}{I(0)} = \frac{k_S k_{fis}^0 (\chi - 1)}{(2k_{fis}^0 + k_S)(\chi k_{fis}^0 + k_S)} \quad (1)$$

where $I(B)$ is the photocurrent as a function of magnetic field strength. Dissociation of the singlet exciton directly into charge is only likely to compete with fission for pentacene molecules directly adjacent to the acceptor. Indeed, reductions in the singlet exciton lifetime of pentacene have been observed in very thin pentacene films (0.6 monolayer) adjacent to a C $_{60}$ layer (10). Thus, we can approximately model pentacene films of varying thickness by changing the effective rate of singlet dissociation in Eq. 1.

Analytically, we can solve for χ at a given value of the magnetic field by noting that the magnitude of δ is maximized when $k_S = k_{fis}^0 \sqrt{2\chi}$. This yields Eq. 2:

$$\chi = \frac{2\delta_{\max}^2 + \delta_{\max} + 1 + 2\sqrt{2\delta_{\max}}\sqrt{\delta_{\max} + 1}}{(\delta_{\max} - 1)^2} \quad (2)$$

The result for χ can be used to directly obtain the triplet yield of singlet fission from

the magnetic field modulation in photocurrent (Eq. 3):

$$\eta_{fis} = \frac{2}{1 + k_S/k_{fis}^0} = \frac{(1 - \delta)\chi - 1 \pm \sqrt{(\delta(\chi + 2) - \chi + 1)^2 - 8\delta^2\chi}}{(\delta + 1)(\chi - 1)} \quad (3)$$

To obtain an independent measure of the yield of singlet fission, we fabricated multiple devices while varying the thickness of pentacene (Fig. 4). For thin layers of pentacene ($d < 5$ nm), we increased the optical absorption by using the multilayer photodetector architecture (4, 26). Photodetectors were measured in reverse bias to improve charge extraction. As a test of generality, both C $_{60}$ and 3,4,9,10-perylene tetracarboxylic bisbenzimidazole (PTCBI) were used as acceptor molecules and found to yield similar results. Devices with thicker layers of pentacene made use of the same device architecture as in Fig. 2. The magnetic field modulation of photocurrent at 0.4 T is shown in Fig. 4A. It peaks at $\delta_{\max} = -(2.7 \pm 0.1)\%$ in 2-nm-thick layers of pentacene sandwiched between acceptor layers. From Eq. 2, we obtain $\chi = 0.85$, identical to the value assumed in (4) on the basis of tetra-cene measurements (27).

In Fig. 4B, we apply Eq. 3 to transform the magnetic field modulation data into the expected yield of triplet excitons from singlet fission. We find that singlet fission is incomplete in pentacene films with thickness $d < 5$ nm, accounting for the relatively low IQE in the photodetector structures. The triplet yield approaches 200% in thicker

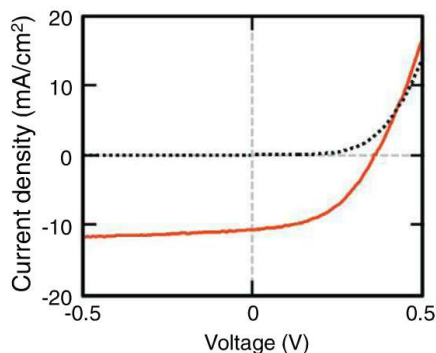
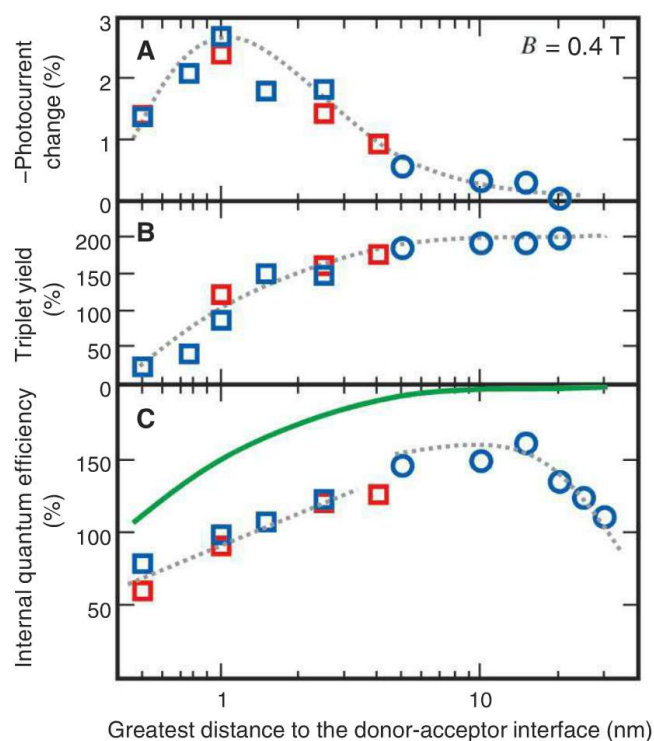


Fig. 3. Current density-voltage characteristic of a pentacene solar cell. The solar cell was measured under dark (black dashed line) or AM1.5G 100 mW/cm 2 (red line) conditions without optical trapping. The power efficiency is $(1.8 \pm 0.1)\%$.

Fig. 4. Triplet yield and IQE as a function of pentacene thickness. (A) The magnetic field-dependent change in photocurrent measured at $B = 0.4$ T as a function of pentacene layer thickness. Square symbols are measured in photodetector structures, and each pentacene layer is sandwiched between C $_{60}$ (blue squares) or PTCBI (red squares) acceptor films. Measurements in the solar cell architecture of Fig. 2A are circles. (B) The triplet yield from singlet exciton fission as obtained from Eq. 3. (C) A comparison of the maximum achievable quantum yield determined from the magnetic field effect (green line) with the internal quantum efficiency as determined from EQE measurements and the calculated optical absorption. The reduction in quantum efficiency observed in thin layers of pentacene is found to originate in incomplete singlet exciton fission. Gray dashed lines are a guide to the eye.



films, providing independent confirmation of the high IQE calculated for the device structure shown in Fig. 2.

The IQE, as evaluated with optical modeling (26), is shown in Fig. 4C and compared to predictions based on the magnetic field effect. The IQE is suppressed in thin layers of pentacene, increases to a maximum for $d \sim 15$ nm, and then is reduced in thicker films. Decreases in IQE for thicker films are presumably due to triplet exciton diffusion limitations and lower-than-unity charge collection efficiency. There are two important conclusions from this IQE comparison. First, the yield of singlet fission can be conveniently determined directly from the normalized change in photocurrent under a magnetic field. A high yield is characterized by a vanishing modulation of photocurrent under a magnetic field. Second, singlet fission in pentacene requires a relatively thick film to minimize losses due to singlet exciton dissociation. Fission is not effective in fine-grained blends of pentacene and fullerene or perylene-based acceptors.

The observation of external quantum yields exceeding 100% in the visible spectrum represents a notable advance in the application of singlet fission to solar cells. Next, fission should be paired with a low-bandgap material that harvests photons below the singlet exciton energy. This could be an organic material (11), inorganic semiconductor nanocrystal (12–14), or a conventional inorganic semiconductor (15). High-quality

contemporary silicon solar cells show an AM1.5 efficiency of $\sim 25\%$ (28); singlet fission materials such as tetracene or rubrene could be integrated with silicon cells to double the photocurrent from high-energy solar photons ($\lambda < 550$ nm), ultimately boosting the efficiency of the silicon cell to more than 30%.

References and Notes

- W. Shockley, H. J. Queisser, *J. Appl. Phys.* **32**, 510 (1961).
- O. E. Semonin *et al.*, *Science* **334**, 1530 (2011).
- M. B. Smith, J. Michl, *Chem. Rev.* **110**, 6891 (2010).
- J. Lee, P. Jadhav, M. A. Baldo, *Appl. Phys. Lett.* **95**, 033301 (2009).
- M. W. Wilson *et al.*, *J. Am. Chem. Soc.* **133**, 11830 (2011).
- J. C. Johnson, T. H. Reilly, A. C. Kanarr, J. van de Lagemaat, *J. Phys. Chem. C* **113**, 6871 (2009).
- C. Jundt *et al.*, *Chem. Phys. Lett.* **241**, 84 (1995).
- H. Marcinia *et al.*, *Phys. Rev. Lett.* **99**, 176402 (2007).
- V. K. Thorsmølle *et al.*, *Phys. Rev. Lett.* **102**, 017401 (2009).
- W. L. Chan *et al.*, *Science* **334**, 1541 (2011).
- P. J. Jadhav, A. Mohanty, J. Sussman, J. Lee, M. A. Baldo, *Nano Lett.* **11**, 1495 (2011).
- P. J. Jadhav *et al.*, *Adv. Mater.* **24**, 6169 (2012).
- B. Ehrler *et al.*, *Nat. Commun.* **3**, 1019 (2012).
- B. Ehrler, M. W. Wilson, A. Rao, R. H. Friend, N. C. Greenham, *Nano Lett.* **12**, 1053 (2012).
- D. L. Dexter, *J. Lumin.* **18–19**, 779 (1979).
- A. Suna, *Phys. Rev. B* **1**, 1716 (1970).
- R. C. Johnson, R. E. Merrifield, *Phys. Rev. B* **1**, 896 (1970).
- S. Yoo, B. Dornier, B. Kippelen, *Appl. Phys. Lett.* **85**, 5427 (2004).
- B. Xu, S. Holdcroft, *Adv. Mater.* **6**, 325 (1994).
- Z.-L. Guan *et al.*, *Org. Electron.* **11**, 1779 (2010).

- Materials and methods are available as supplementary materials on Science Online.
- F. Monestier *et al.*, *J. Appl. Phys.* **102**, 034512 (2007).
- P. D. Reusswig, D. N. Congreve, N. J. Thompson, M. A. Baldo, *Appl. Phys. Lett.* **101**, 113304 (2012).
- S.-B. Rim, S. Zhao, S. R. Scully, M. D. McGehee, P. Peumans, *Appl. Phys. Lett.* **91**, 243501 (2007).
- K. Tvingstedt, V. Andersson, F. Zhang, O. Inganäs, *Appl. Phys. Lett.* **91**, 123514 (2007).
- P. Peumans, A. Yakimov, S. R. Forrest, *J. Appl. Phys.* **93**, 3693 (2003).
- H. Bouchriha, V. Ern, J. L. Fave, C. Guthmann, M. Schott, *Phys. France* **39**, 257 (1978).
- M. A. Green, *Prog. Photovolt. Res. Appl.* **17**, 183 (2009).
- N. Sato, Y. Saito, H. Shinohara, *Chem. Phys.* **162**, 433 (1992).
- A. Kahn, N. Koch, W. Gao, *J. Polym. Sci. Part B* **41**, 2529 (2003).
- J. Hwang, A. Wan, A. Kahn, *Mater. Sci. Eng. Rep.* **64**, 1 (2009).

Acknowledgments: This work was supported as part of the Center for Excitronics, an Energy Frontier Research Center funded by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under award no. DE-SC0001088 (MIT). D.N.C. was partially supported by the NSF Graduate Research Fellowship under grant 1122374. MIT has filed a provisional application for patent based on this technology that names D.N.C., N.J.T., and M.A.B. as inventors.

Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6130/334/DC1

Materials and Methods

Supplementary Text

Figs. S1 to S5

References (32–37)

20 November 2012; accepted 28 January 2013

10.1126/science.1232994

Multicompartment Mesoporous Silica Nanoparticles with Branched Shapes: An Epitaxial Growth Mechanism

Teeraporn Suteewong,^{1,2*} Hiroaki Sai,^{1*} Robert Hovden,³ David Muller,^{3,4} Michelle S. Bradbury,² Sol M. Gruner,^{4,5,6} Ulrich Wiesner^{1†}

Mesoporous nanomaterials have attracted widespread interest because of their structural versatility for applications including catalysis, separation, and nanomedicine. We report a one-pot synthesis method for a class of mesoporous silica nanoparticles (MSNs) containing both cubic and hexagonally structured compartments within one particle. These multicompartment MSNs (mc-MSNs) consist of a core with cage-like cubic mesoporous morphology and up to four branches with hexagonally packed cylindrical mesopores epitaxially growing out of the cubic core vertices. The extent of cylindrical mesostructure growth can be controlled via a single additive in the synthesis. Results suggest a path toward high levels of architectural complexity in locally amorphous, mesostructured nanoparticles, which could enable tuning of different pore environments of the same particle for specific chemistries in catalysis or drug delivery.

Mesoporous silica materials have attracted widespread interest because of their versatility in pore structure, surface chemistry, and macroscopic form (particles, coatings, or bulk materials) (1, 2). A variety of mesostructures have been explored, including hexagonal, cage-like cubic, cubic bicontinuous, and platelet ordered structures as well as, most recently, dodecagonal quasicrystalline structures (3, 4). Mesoporous silica nanoparticles (MSNs) are of particular

interest owing to their large surface area and pore volume, the ability to functionalize their outer and/or inner surfaces, and the tunability of their pore geometry (5, 6). Multicompartment nanoparticles have been developed from self-assembling designer macromolecules (7–9). Such materials have been compared with cells as the paradigm for a multicompartment system, in which different units (e.g., nucleus or organelles) have different well-defined functions. In potential drug delivery

applications for these materials, each compartment could be tuned to carry different and incompatible components (7).

Despite the existing library of geometrical variations of the silica pore mesostructure, the synthesis of analogous multicompartment MSNs (mc-MSNs) remains a challenge, partly because of the lack of growth mechanisms enabling pore structure control in different compartments of the same particle. Identifying such growth mechanisms would enable access to locally amorphous, mesoscopically ordered nanoparticles with a high degree of architectural complexity yet highly controlled shapes and assemblies.

The field of solution-grown semiconductor nanoparticle synthesis provides a possible clue to the creation of such architectures. Such nanoparticles may have polymorphic atomic structures that are epitaxially attached at the interface from a core, leading to branched inorganic nanostructures with well-defined and characteristic shapes

¹Department of Materials Science and Engineering, Cornell University, Ithaca, NY 14853, USA. ²Department of Radiology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA. ³School of Applied and Engineering Physics, Cornell University, Ithaca, NY 14853, USA. ⁴Kavli Institute at Cornell for Nanoscale Science, Ithaca, NY 14853, USA. ⁵Department of Physics, Cornell University, Ithaca, NY 14853, USA. ⁶Cornell High Energy Synchrotron Source (CHESS), Cornell University, Ithaca, NY 14853, USA.

*These authors contributed equally to this work.
†Corresponding author. E-mail: ubw1@cornell.edu

such as tetrapods and dendrimers (10–12). Rather than epitaxy from atomic structures, mesostructural epitaxy exists in mesoporous silica—that is, for various cubic lattices as well as between $Pm\bar{3}n$ and two-dimensional (2D) hexagonal lattices (13–18). We investigated how such mesostructural epitaxy can be used in low molar mass surfactant coassembly to generate mc-MSNs with branched shapes in which the branches exhibit pore geometries different from that of the core, based on different mesostructural lattices.

Highly aminated cubic MSNs with cage-like pore structure consistent with $Pm\bar{3}n$ symmetry were synthesized at room temperature from mixed organosilane precursors via a one-pot, hexadecyltrimethylammonium bromide (CTAB)

surfactant-directed sol-gel synthesis, with variations in ethyl acetate concentration [EtOAc] (19–21). When [EtOAc] was increased, mesoporous silica branches with hexagonal cylinder pore structure grew from specific facets of the cubic MSN cores (Fig. 1, A and B). Bright-field transmission electron microscopy (BF-TEM) images indicated that these two parts or “compartments” of the nanoparticles have a well-defined structural relation when viewed along the $[110]$ zone axis (Fig. 1A). Furthermore, some particles possessed multiple branches (Fig. 1B). High-angle annular dark-field scanning TEM (HAADF-STEM) images of mc-MSNs along the $[100]$ zone axis (Fig. 1C) showed the characteristic four-fold internal structure consistent with the faceted overall particle

shape. Fast Fourier transform (FFT) patterns of the HAADF-STEM image exhibited spots for the (110) , (200) , and (210) planes, consistent with $Pm\bar{3}n$ symmetry (Fig. 1D). Closer inspection of the particle in Fig. 1C revealed the corresponding characteristic four-fold internal patterns (Fig. 1E). Figure 1F depicts the corresponding model schematic viewed from a similar angle as in Fig. 1C. Although the size of micellar pores relative to the overall particle is modified in this model to better show the internal structure, the external shape of the particle in Fig. 1C is consistent with the model in Fig. 1F, where the cube is truncated at its corners with (111) planes and its sides with (110) planes. Occasionally the branches were also observed to be faceted along the $(10\bar{1}0)$ planes (fig. S1),

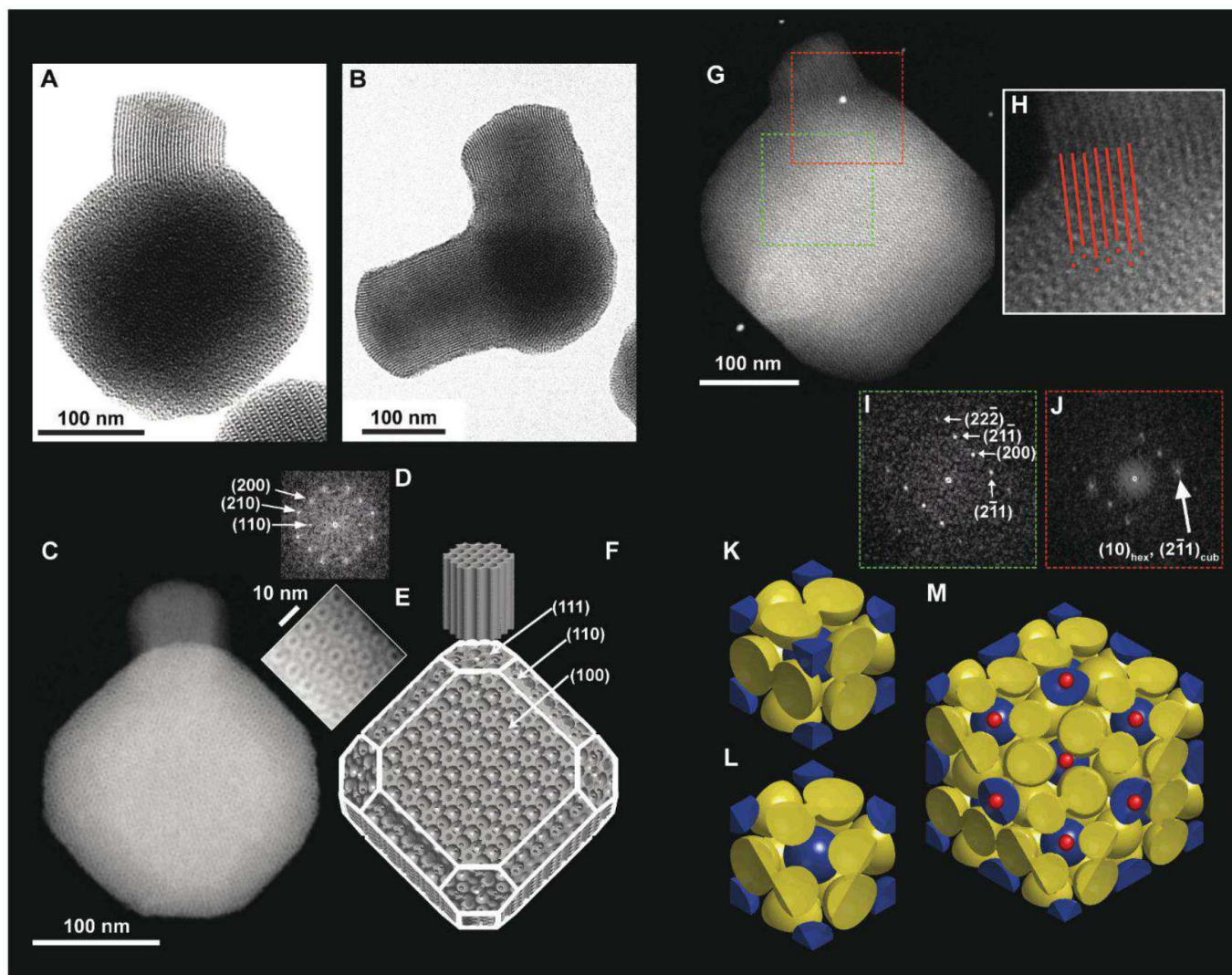


Fig. 1. Geometrical description of mc-MSNs. (A) BF-TEM image of a branch with hexagonal pore structure emanating from one corner of a core with cubic pore structure. (B) BF-TEM image showing two branches emanating from two corners of the core. (C) HAADF-STEM image of (100) projection of the core. (D) FFT of the entire cubic core part of the image in (C). (E) Magnified image of the top right edge of the particle in (C). (F) Schematics of a model mc-MSN. Miller indices for some of the representative facets are provided as a visual guide. (G) HAADF-STEM image of a mc-MSN exhibiting the (110) projection of the core. (H) Magnified image of the connecting region between the core and

branch in (G), with red lines and dots indicating projected vacancy positions to demonstrate the structural registry/epitaxy. (I) FFT of the cubic core region in (G) (green box). (J) FFT of the connected region in (G) (red box). (K to M) Model visualization of the epitaxial relationship of mesopores at the interface of (111) $Pm\bar{3}n$ cubic and (0001) $P6mm$ hexagonal planes. (K) Unit cell of the $Pm\bar{3}n$ cage-like structure exhibiting body-centered cubic lattice micelles (blue spheres) and pairs of micelles on the faces (yellow spheres). (L) (111) plane cut of a single unit cell. (M) (111) plane cut of a $2 \times 2 \times 2$ lattice, additionally showing the positions of expected hexagonal channels as small red dots.

coincident with (110) planes on the core, which provided a basis for determining the facets in the model. When HAADF-STEM images were obtained along the $[110]$ zone axis (Fig. 1G) of the core, the internal linear pore structure of the branch was clearly observed. Figure 1H shows a magnified image of the connecting region between the core and the branch. Red lines representing the cylindrical pores of the branch are in registry with the micellar pores of the core depicted as red dots, visually supporting an epitaxial relationship between the mesostructures of the two compartments.

FFT of the core region showed spots corresponding to the $Pm\bar{3}n$ peak positions with the (110) zone axis (Fig. 1I), whereas FFT of the connected region showed an overlap of sharp (211) spots from the cubic lattice and diffuse (10) spots from the 2D hexagonal lattice, also consistent with an epitaxial relationship between the two structures (Fig. 1J). This epitaxial relationship can be modeled by a sphere-to-rod transition of micelles, as illustrated in Fig. 1, K to M. A $Pm\bar{3}n$ cage-like cubic structure can be composed of a body-centered cubic arrangement of micelles plus pairs of micelles on each cubic face (Fig. 1K). Sectioning the unit cell at the (111) plane, each blue micelle resides on top of a set of three yellow micelles, making a locally layered order (Fig. 1L). Figure 1M is a top-view schematic showing where the

2D hexagonal channels are placed with respect to the (111) cubic planes; the hexagonal lattice of the blue micelles is in registry with the hexagonally ordered channels, consistent with the observations shown in Fig. 1H and highlighted by the red lines and dots.

The relative amount of hexagonal versus cubic mesostructure could be controlled by increasing $[EtOAc]$ in the initial mixture (Fig. 2). Low-magnification TEM images of MSNs synthesized from 91, 274, and 457 mM $EtOAc$ (Fig. 2, A, D, and G, respectively) show hexagonally ordered branches only at elevated concentrations (Fig. 2A shows only cubic MSNs). The length of these hexagonally ordered branches increased with increasing $[EtOAc]$ (compare Fig. 2, D and G). Most of the mc-MSNs synthesized from 274 mM $EtOAc$ had short branches with diameters equal to or smaller than the core size (Fig. 2D). In the sample synthesized from 457 mM $EtOAc$, these branches grew to rods as long as 2 μm , often in a nonstraight fashion (Fig. 2G). Higher-magnification images show that the nanoparticles synthesized from 274 mM $EtOAc$ (Fig. 2E) consist of a branch attached to a core with cage-like cubic structure. At 457 mM $EtOAc$ (Fig. 2H), for most of the rods we could not find a core portion, suggesting structural transformation from cubic to hexagonal mesostructure.

Small-angle x-ray scattering (SAXS) patterns (Fig. 2, C, F, and I) of these samples averaging over macroscopic material volumes corroborate the local TEM observations, in that the relative intensities of the peaks consistent with $P6mm$ 2D hexagonal symmetry increased at the expense of those consistent with $Pm\bar{3}n$ symmetry. The cubic MSN sample synthesized from 91 mM $EtOAc$ showed reflections consistent with $Pm\bar{3}n$ symmetry and a unit cell size of 9.65 nm (Fig. 2C) (19). At 274 mM $EtOAc$, reflections consistent with 2D hexagonal symmetry and a unit cell size of 4.80 nm were superposed with reflections consistent with $Pm\bar{3}n$ symmetry and a unit cell size of 10.5 nm (Fig. 2F). The (211) reflection for the cubic lattice coincided closely with the (10) reflection for the hexagonal lattice, with lattice mismatch of $\sim 3\%$, corroborating an epitaxial relation between the two lattices. The pore-to-pore spacings for the hexagonal branches in the mc-MSNs synthesized from 274 mM $EtOAc$ were 8% larger than those for purely hexagonal MSNs synthesized in the absence of (3-aminopropyl)triethoxysilane (APTES) and were 3 to 4% larger than those synthesized in the presence of varying amounts of APTES (fig. S2) (20, 22). On the other hand, the (211) spacing of the cubic core was 4.95 nm, indicating that the 2D hexagonal lattice is stretched to accommodate for the lattice mismatch. At 457 mM $EtOAc$, reflections consistent with $Pm\bar{3}n$ had almost disappeared, leaving mostly those consistent with 2D hexagonal symmetry and a pore-to-pore spacing of 4.86 nm (Fig. 2I). The increase in spacing as a function of $[EtOAc]$ for both cubic and hexagonal structures (fig. S2) is likely associated with swelling of the hydrophobic micelle cores with $EtOAc$ molecules.

To establish a structure-property relationship for these mc-MSNs, we performed nitrogen sorption measurements (Fig. 3). All three samples showed characteristic type IV isotherms, with the capillary condensation of mesopores occurring at nitrogen partial pressure around 0.3. The mc-MSNs containing more hexagonal pores (i.e., synthesized from higher $[EtOAc]$) exhibited a sharper mesopore condensation behavior, as reflected in the pore size distribution calculated using nonlocal density functional theory (NLDFT) (Fig. 3, inset). The cubic MSN showed a broad distribution of pore sizes centered at 3.6 nm and extending up to 4.5 nm; mc-MSNs with increasing numbers of hexagonal pores exhibited narrower pore size distributions centered around 3 to 4 nm. This sorption behavior is consistent with the cage-like pore geometry with constricted windows between the cages in the cubic core and the straight channel geometry in the hexagonal branches (23).

The above trend in MSN structure as a function of $[EtOAc]$ sheds light on a possible growth mechanism of hexagonal branches emanating from the cubic cores. In basic conditions, $EtOAc$ is known to hydrolyze over time into ethanol and acetic acid, which quenches an equimolar amount of base and thus lowers the pH of the reaction

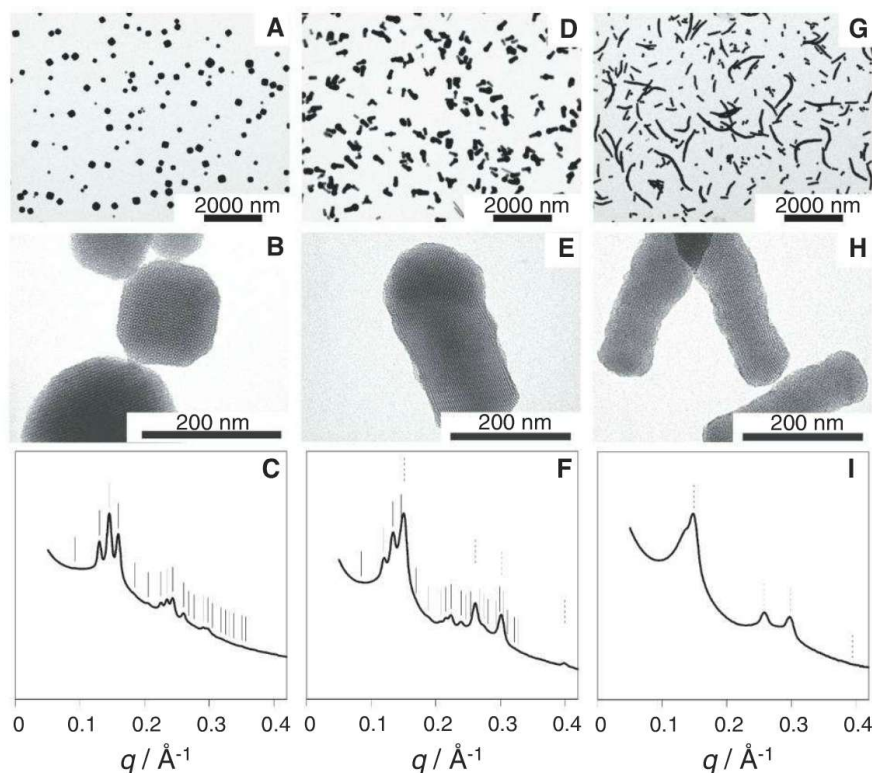


Fig. 2. TEM images and SAXS patterns of aminated MSNs synthesized with varying $[EtOAc]$. (A, D, and G) Low-magnification TEM images of MSNs prepared from (A) 91 mM, (B) 274 mM, and (C) 457 mM $EtOAc$. (B, E, and H) Higher-magnification TEM images of the MSNs in (A), (D), and (G). (C, F, and I) The corresponding SAXS patterns (q denotes the scattering vector magnitude, defined as $q = 4\pi \sin \theta / \lambda$, where 2θ is the total scattering angle and λ is the x-ray wavelength). In the SAXS patterns, expected peak positions from cubic and hexagonal lattices are indicated by solid and dotted lines, respectively. Data for 91 mM $EtOAc$ are adapted from (19).

mixture (24). At room temperature, the kinetic constant of ester hydrolysis is $5.4 \text{ liters mol}^{-1} \text{ min}^{-1}$ (25). Increasing [EtOAc] from 91 mM to 457 mM while keeping the concentration of base (ammo-

nium hydroxide and APTES) constant at $\sim 200 \text{ mM}$ imposes two effects on the solution pH (fig. S3). First, it induces an increase in the hydrolysis rate and thus in the rate of pH drop. Second, it lowers

the final pH of the solution asymptotically with respect to [EtOAc], because the hydrolysis reaction slows as the hydroxide ions are quenched. As pH decreases below the negative logarithm of the acid dissociation constant ($\text{p}K_a$) of APTES, the predominant state of the amine group of APTES becomes positively charged, which repels against the charged head groups of the structure-directing CTAB cations. This change in the charge state of APTES may induce a structural change in the CTAB micelles. Although at high pH some of the uncharged APTES molecules can intercalate between CTAB head groups, charge repulsion prevents this swelling at lower pH. The resulting change in micelle curvature has been suggested as the main factor driving a morphological change from cage-like cubic to hexagonal, in agreement with the observed transition from a cubic core to hexagonal branches growing off of this core in the present study (23, 26). The room-temperature synthesis protocol used here enables fine-tuning of the sol-gel kinetics against pH change, providing control over the mc-MSN morphology. In energy-dispersive spectroscopy-derived elemental maps, we did not observe substantial variations in the amine content for the cubic versus hexagonal parts of the nanoparticles (fig. S4), suggesting high inclusion of APTES in both cubic and hexagonal pore walls.

Nucleation of hexagonally mesostructured compartments on the cubic MSN cores can occur on multiple facets of the cubic core, most evident

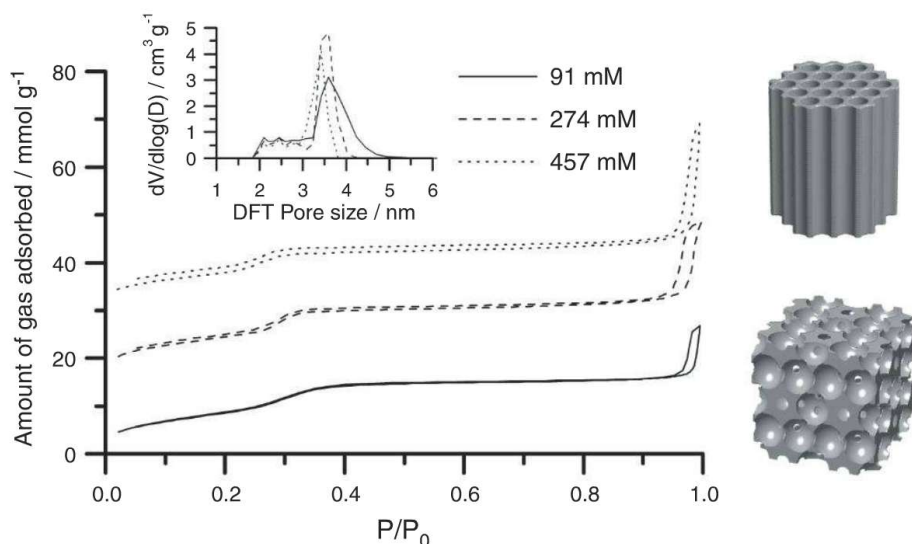


Fig. 3. Nitrogen sorption isotherms of MSNs synthesized with varying [EtOAc]. Isotherms for mc-MSNs prepared from 274 mM and 457 mM EtOAc are offset along the y axis by 15 and 30 mmol/g, respectively (P/P_0 denotes the nitrogen partial pressure). The inset shows pore size distributions obtained from NLDFT calculations based on the respective absorption branches. The models on the right provide a direct comparison of the pore structures of hexagonal and cubic lattices.

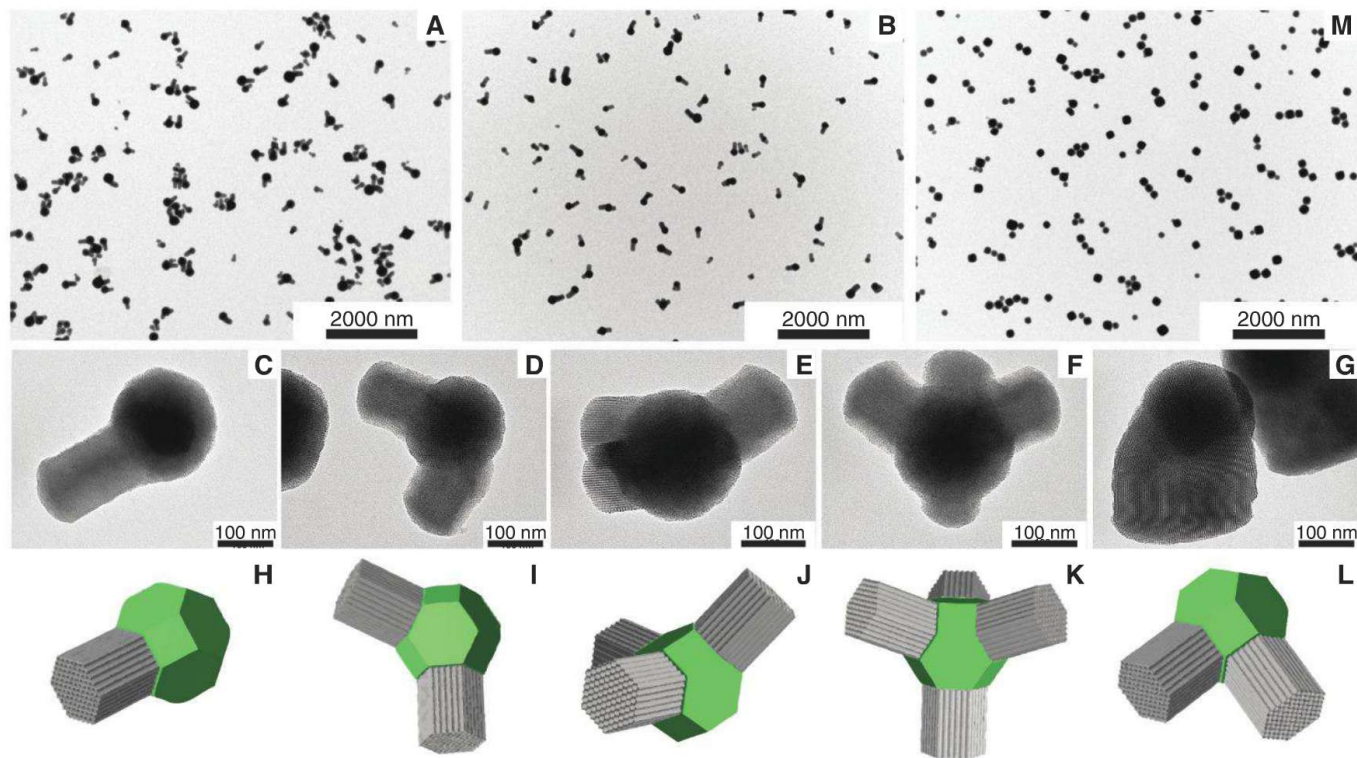


Fig. 4. TEM images and models of mc-MSNs with complex mesostructures. (A and B) Low-magnification TEM images of mc-MSNs prepared from (A) 137 mM and (B) 183 mM EtOAc. (C to G) High-magnification TEM images of mc-MSNs in the 183 mM EtOAc batch with (C) one arm, (D) two arms, (E) three arms, (F) four arms, and (G) two arms merged into one.

(H to L) Models corresponding to the images in (C) to (G). Cores are shown as green truncated cubes; branches are represented as gray columns. (M) Low-magnification TEM image of mc-MSNs where additional silane precursors (one-half of the initial amount) were injected after 30 min of aging time (21).

at intermediate [EtOAc]. At 137 mM and 183 mM EtOAc, although most particles still showed only one branch per core, a fraction had one, two, three, and even four branches (Fig. 4, A and B). Close TEM examination of these mc-MSNs revealed that the angles at which the hexagonally structured branches grew were in agreement with models in which hexagonal branches grow in $\langle 111 \rangle$ directions off of the core, consistent with the epitaxial relationship (compare TEM images with corresponding models in Fig. 4, C to F and H to K, respectively). No MSN with more than four branches was observed in our samples, although we did observe some mc-MSNs in which hexagonal branches that grew in two distinct directions merged into one branch with a large diameter (compare TEM image with model in Fig. 4, G and L). On a truncated cubic structure, there are eight $\langle 111 \rangle$ equivalent surfaces corresponding to the number of cube vertices, giving eight equivalent sites for hexagonal branches to form and grow. As a particular branch grows, it may deplete the available silica in its direct vicinity, thus preventing growth of another branch next to it. This is one possible explanation for why the vast majority of observed branches had grown on non-nearest $\langle 111 \rangle$ core surfaces and why the maximum number of branches observed was only four. We further hypothesize that when two nearest neighboring sites nucleate hexagonal branches, geometrical crowding joins the neighboring nuclei as they grow. Such merging and overgrowth of hexagonal rods also reduces the number of rods per particle and increases the rod diameter relative to the core particle size. This may be what leads to the observed structures in the 457 mM EtOAc sample (Fig. 2G).

Finally, to access higher-level architectures, we performed mc-MSN syntheses in a two-step fashion by adding further monomers at different time points of the reaction (21). The TEM results in Fig. 4M, displaying a high number of dimers (two cubes) and trimers (three cubes) connected via bridges, are encouraging. They suggest that further tuning of reaction conditions holds promise for achieving even more complex architectures—for example, through switching back and forth between cubic-to-hexagonal and hexagonal-to-cubic transitions—similar to what has been observed for semiconductor nanoparticles or colloids with valence (11, 27).

References and Notes

1. T. Yanagisawa, T. Shimizu, K. Kuroda, C. Kato, *Bull. Chem. Soc. Jpn.* **63**, 988 (1990).
2. C. T. Kresge, M. E. Leonowicz, W. J. Roth, J. C. Vartuli, J. S. Beck, *Nature* **359**, 710 (1992).
3. F. Hoffmann, M. Cornelius, J. Morell, M. Fröba, *Angew. Chem. Int. Ed.* **45**, 3216 (2006).
4. C. H. Xiao, N. Fujita, K. Miyasaka, Y. Sakamoto, O. Terasaki, *Nature* **487**, 349 (2012).
5. M. Vallet-Regí, F. Balas, D. Arcos, *Angew. Chem. Int. Ed.* **46**, 7548 (2007).
6. I. I. Slowing, J. L. Vivero-Escoto, C. W. Wu, V. S. Lin, *Adv. Drug Deliv. Rev.* **60**, 1278 (2008).
7. Z. B. Li, E. Kesselman, Y. Talmon, M. A. Hillmyer, T. P. Lodge, *Science* **306**, 98 (2004).
8. H. G. Cui, Z. Y. Chen, S. Zhong, K. L. Wooley, D. J. Pochan, *Science* **317**, 647 (2007).
9. R. Chandrawati, M. P. van Koeveden, H. Lomas, F. Caruso, *J. Phys. Chem. Lett.* **2**, 2639 (2011).
10. L. Manna, D. J. Milliron, A. Meisel, E. C. Scher, A. P. Alivisatos, *Nat. Mater.* **2**, 382 (2003).
11. Y. Yin, A. P. Alivisatos, *Nature* **437**, 664 (2005).
12. D. V. Talapin et al., *Nano Lett.* **7**, 2951 (2007).
13. P. Srinivasu, S. Lim, Y. Kubota, T. Tatsumi, *Catal. Today* **111**, 379 (2006).
14. L. Han, Y. Sakamoto, S. Che, O. Terasaki, *Chemistry* **15**, 2818 (2009).

15. R. Attluri, Z. Bacsik, N. Hedin, A. E. Garcia-Bennett, *Micropor. Mesopor. Mater.* **133**, 27 (2010).
16. L. Han, K. Miyasaka, O. Terasaki, S. N. Che, *J. Am. Chem. Soc.* **133**, 11524 (2011).
17. S. N. Che, S. Kamiya, O. Terasaki, T. Tatsumi, *J. Am. Chem. Soc.* **123**, 12089 (2001).
18. S. Kamiya, H. Tanaka, S. Che, T. Tatsumi, O. Terasaki, *Solid State Sci.* **5**, 197 (2003).
19. T. Suteewong et al., *J. Am. Chem. Soc.* **133**, 172 (2011).
20. T. Suteewong et al., *Chem. Mater.* **24**, 3895 (2012).
21. See supplementary materials on Science Online.
22. T. Suteewong et al., *J. Mater. Chem.* **20**, 7807 (2010).
23. R. Attluri, Y. Sakamoto, A. E. Garcia-Bennett, *Langmuir* **25**, 3189 (2009).
24. G. Schulz-Ekloff, J. Rathousky, A. Zukal, *Int. J. Inorg. Mater.* **1**, 97 (1999).
25. L. W. Dittert, T. Higuchi, *J. Pharm. Sci.* **52**, 852 (1963).
26. M. Ogura, H. Miyoshi, S. P. Naik, T. Okubo, *J. Am. Chem. Soc.* **126**, 10937 (2004).
27. Y. Wang et al., *Nature* **491**, 51 (2012).

Acknowledgments: Supported by NSF single-investigator grant DMR-1104773 and by a Partnership for Research and Education in Materials (PREM) program at Norfolk State University through NSF grant DMR-1205457. We thank L. Estroff, R. Dieckmann, and K. Hur (Cornell University) for discussions on multiple branch growth and K. W. Tan (Cornell University) for assistance with EM. This work made use of the TEM and STEM supported by the Cornell Center for Materials Research with support from the NSF Materials Research Science and Engineering Centers program (DMR-1120296), and CHESS, which is supported by the NSF and the National Institute of General Medical Sciences under NSF award DMR-0936384. T.S. is grateful for a Thai Government Scholarship under the Ministry of Science and Technology.

Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6130/337/DC1
Materials and Methods
Figs. S1 to S4
Reference (28)

11 October 2012; accepted 1 February 2013
10.1126/science.1231391

Reorganization of Southern Ocean Plankton Ecosystem at the Onset of Antarctic Glaciation

Alexander J. P. Houben,^{1,*†} Peter K. Bijl,¹ Jörg Pross,^{2,3} Steven M. Bohaty,⁴ Sandra Passchier,⁵ Catherine E. Stickley,⁶ Ursula Röhl,⁷ Saiko Sugisaki,⁸ Lisa Tauxe,⁸ Tina van de Flierdt,⁹ Matthew Olney,¹⁰ Francesca Sangiorgi,¹ Appy Sluijs,¹ Carlota Escutia,¹¹ Henk Brinkhuis,¹ and the Expedition 318 Scientists‡

The circum-Antarctic Southern Ocean is an important region for global marine food webs and carbon cycling because of sea-ice formation and its unique plankton ecosystem. However, the mechanisms underlying the installation of this distinct ecosystem and the geological timing of its development remain unknown. Here, we show, on the basis of fossil marine dinoflagellate cyst records, that a major restructuring of the Southern Ocean plankton ecosystem occurred abruptly and concomitant with the first major Antarctic glaciation in the earliest Oligocene (~33.6 million years ago). This turnover marks a regime shift in zooplankton-phytoplankton interactions and community structure, which indicates the appearance of eutrophic and seasonally productive environments on the Antarctic margin. We conclude that earliest Oligocene cooling, ice-sheet expansion, and subsequent sea-ice formation were important drivers of biotic evolution in the Southern Ocean.

Long-term cooling during the middle and late Eocene [~49 to 34 million years ago (Ma)] led to the inception of continental-

scale glaciation of Antarctica during the Eocene-Oligocene Transition (EOT, 34 to 33.5 Ma) (1, 2). The EOT culminated in a pronounced positive

shift in the oxygen isotope ratios of marine biogenic calcite during Oligocene isotope event 1 (O1; 33.7 Ma) (3, 4). Direct evidence for

¹Department of Earth Sciences, Laboratory of Palaeobotany and Palynology (LPP), Faculty of Geosciences, Utrecht University, Budapestlaan 4, 3584 CD Utrecht, Netherlands. ²Paleo-environmental Dynamics Group, Institute of Geosciences, Goethe University Frankfurt, Altenhöferallee 1, 60438 Frankfurt, Germany. ³Biodiversity and Climate Research Centre, Senckenberganlage 25, 60325 Frankfurt, Germany. ⁴Ocean and Earth Science, University of Southampton, National Oceanography Centre, European Way, Southampton SO14 3ZH, UK. ⁵Department of Earth and Environmental Studies, Montclair State University, 1 Normal Avenue, Montclair, NJ 07043, USA. ⁶Department of Geology, University of Tromsø, Breivika, N-9037 Tromsø, Norway. ⁷MARUM Center for Marine Environmental Sciences, University of Bremen, Leobener Straße, 28359 Bremen, Germany. ⁸Scripps Institution of Oceanography, University of California at San Diego, 8635 Kennel Way, La Jolla, CA 92093-0220, USA. ⁹Department of Earth Science and Engineering, Imperial College London, Exhibition Road, SW7 2AZ, London, UK. ¹⁰Department of Geology, University of South Florida, 4202 East Fowler Avenue, Tampa, FL 33620, USA. ¹¹Instituto Andaluz de Ciencias de la Tierra, Avenida de las Palmeras, 4, 18100 Armilla (Granada), Spain.

*Corresponding author. E-mail: Alexander.Houben@TNO.nl

†Present address: Netherlands Organization for Applied Scientific Research (TNO), Princetonlaan 6, 3584 CB Utrecht, Netherlands.

‡The complete list of authors and affiliations appears in the supplementary materials.

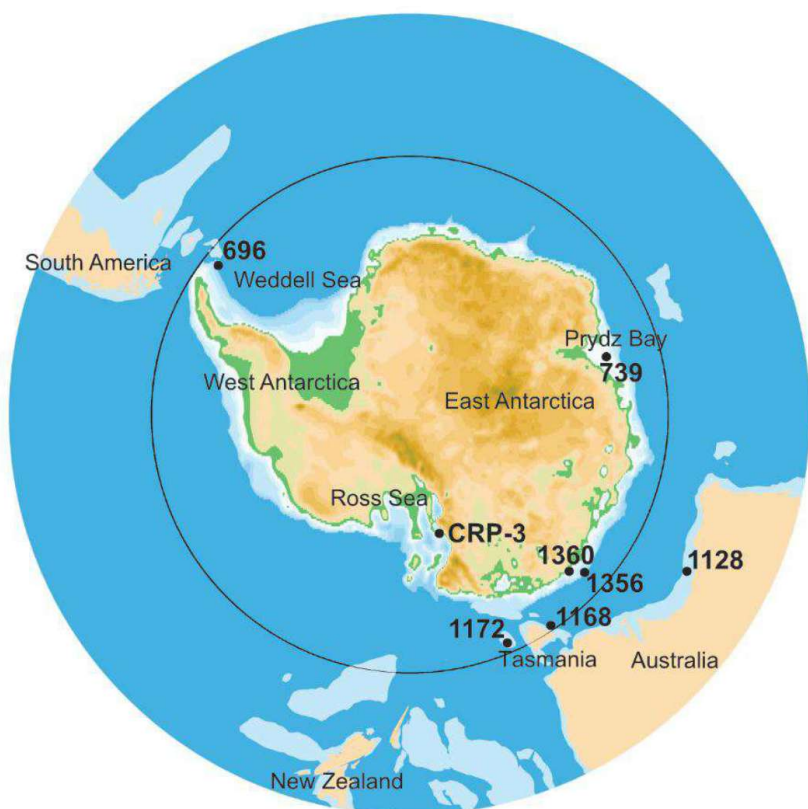


Fig. 1. Antarctic topography [modified after (33), maximum topography model], circum-Antarctic paleogeography at 34 Ma (34) and the location of sites considered in this study. Light blue areas represent shelf environments; green areas represent Antarctic lowland areas. The black circle indicates 60°S.

continental-scale glaciation of Antarctica at O11 includes the appearance of ice-rafted debris and physically weathered clay minerals at sites along the Antarctic margin (5, 6). Numerical climate model simulations suggest that partial to full-scale glaciation of Antarctica occurred after a reduction in atmospheric CO₂ concentrations and a series of low-amplitude orbital variations in solar insolation that resulted in relatively cool austral summer temperatures (7). Such conditions, as well as continental ice, were also likely required for seasonal sea-ice formation (7). As a consequence, the establishment of Antarctic glaciation should have substantially impacted circum-Antarctic Southern Ocean ecosystems, and associated biotic changes are potentially reflected in the fossil record in conjunction with the O11 climate event.

The present-day circum-Antarctic seas are home to distinct plankton communities that are characterized by a high degree of endemism and high seasonal productivity as a result of steep seasonal gradients in temperature, salinity, light availability, and nutrient concentrations associated with sea-ice cover (8, 9). Diatoms and *Phaeocystis* (unicellular, eukaryotic photosynthetic algae) are the dominant primary producers of these communities (9, 10). Whereas the latter does not preserve in the geological record, fossil diatom skeletons composed of silica are routinely used to reconstruct Antarctic sea-ice history for the recent past (11). However, diatom-based sea-ice reconstructions for the Eocene-Oligocene time interval

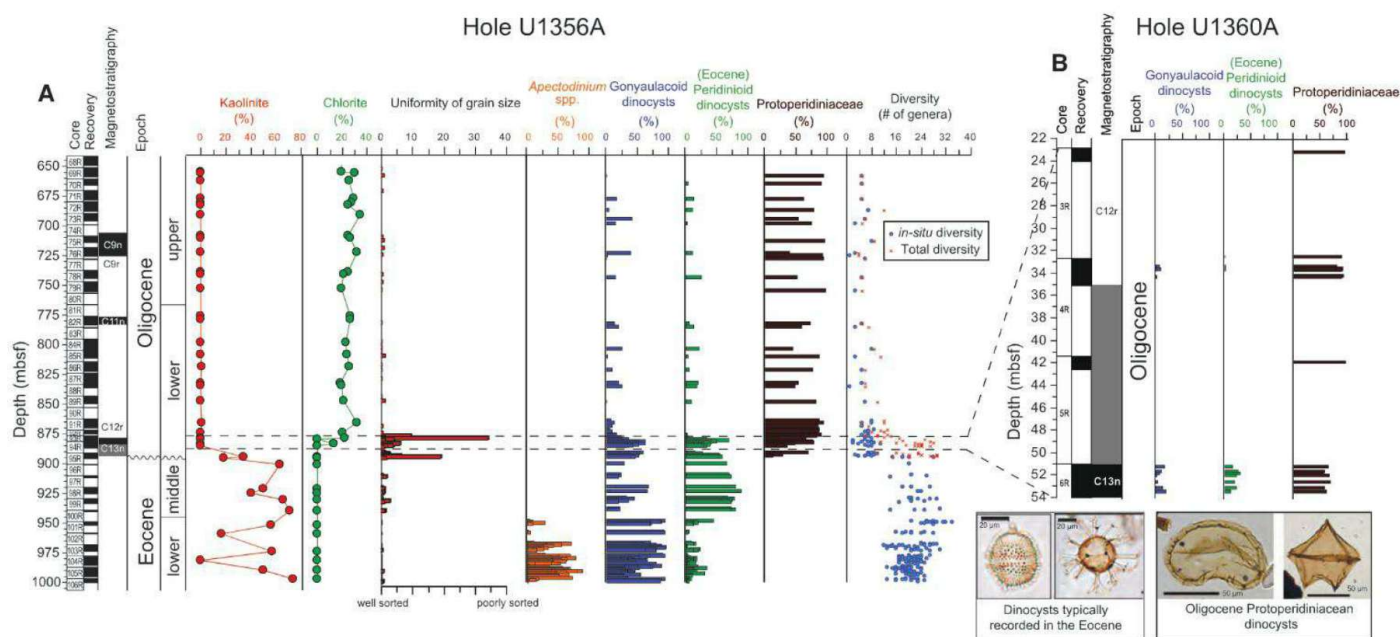


Fig. 2. The Eocene-Oligocene section recovered in IODP hole U1356A (A) and the lower Oligocene section from IODP hole U1360A (B). A shift in clay mineralogy in the lowermost Oligocene of site U1356A indicates the initiation of a physical (i.e., glacial) weathering regime. The uniformity coefficient of grain size—a measure of the sorting of the sediments, with large numbers (>4) indicating poorly sorted sediments—increases episodically in the lower Oligocene, suggesting glacial activity. The magnetostratigraphic interpretations are derived from (19) and as presented in the supplementary text; gray

areas indicate uncertain magnetic polarity. Lower to middle Eocene dinocyst assemblages are diverse (expressed by number of genera), with subtropical components (*Apectodinium*) and autotrophic (gonyaulacoid taxa) and Eocene heterotrophic (peridinioid dinocysts) representatives (supplementary text). We consider the majority of non-protoperidiniacean dinocysts in the lower Oligocene succession reworked (translucent colored bars; see also supplementary text). Photomicrographs of typical Eocene and Oligocene dinocyst taxa are depicted in the lower right corner.

are hampered by poor diatom preservation in Antarctic margin sediments as well as the poorly resolved paleoecology of many fossil taxa (12). Therefore, other microfossil groups must be used to investigate the timing of the installation of these ecosystems and how the development of Antarctic plankton communities was linked to cooling and glaciation in the geological past.

Although dinoflagellates are not a major group in terms of biomass, many heterotrophic species are abundant in the areas of high seasonal productivity in the modern Southern Ocean (13, 14) (supplementary text) and are closely associated with primary algal producers, including diatoms (13). Fortunately, many dinoflagellate taxa also produce distinct organic fossilizable cysts as part of their life cycle, and these dinocysts are preserved in high-latitude sedimentary sequences. In the Eocene, before the onset of Antarctic glaciation, dinocysts are found in great diversity and have been used to reconstruct environmental change in high-latitude environments (15, 16). Hence, dinocyst assemblage analysis of sediment cores is a valuable tool for evaluating circum-Antarctic plankton ecosystem change across the EOT. Yet, such evaluation has been previously hampered by the absence of well-dated records from the Antarctic margin.

Integrated Ocean Drilling Program (IODP) Expedition 318 recovered a Paleogene succession of marine sediments off Wilkes Land, East Antarctica (17). Deep-marine sediments spanning most of the Oligocene were recovered at IODP site U1356 on the continental rise, whereas shallow-marine sediments of early Oligocene age were recovered at IODP site U1360 (Fig. 1). In IODP hole U1356A, a hemipelagic succession of Oligocene claystones and siltstones was recovered

between 894.68 and 422.19 m below the sea floor (mbsf). The base of this section is approximately correlative to the Oi1 event, as indicated by the presence of the marker dinocyst *Malvinia escutiana* (18) and the identification of magnetostratigraphic C13n (19). An ~12-million-year-long hiatus separates the middle Eocene and lower Oligocene sections at Site U1356 (supplementary text) (19). This hiatus marks a change from clay mineral assemblages dominated by kaolinite and smectite, which formed under relatively warm and humid conditions, to abundant chlorite and illite group minerals, which are indicative of physical weathering by glacial and periglacial processes (17). In hole U1360A, clast-bearing glaciomarine sediments were recovered between 54 and 23 mbsf (Fig. 2). These strata were deposited between ~33.6 and ~32 Ma (supplementary text, and they span or immediately postdate Oi1 (17, 19). We generated dinocyst assemblage and sedimentological and geochemical data from holes U1356A and U1360A that document the environmental and ecosystem evolution on the Wilkes Land margin associated with the establishment of the Antarctic cryosphere (20). These data are complemented with qualitative information derived from pyritized siliceous microfossils [e.g., diatoms (supplementary text)] and supporting dinocyst assemblage data from five additional Southern Ocean drillcores that span the EOT (figs. S2, S4, and S5 and supplementary text).

At continental rise site U1356 (Fig. 1), early and middle Eocene (53 to 45 Ma) dinocyst assemblages are highly diverse and are composed of both auto- and heterotrophic dinoflagellate taxa (Fig. 2). In sharp contrast, earliest Oligocene assemblages at sites U1356 and U1360 are low in

diversity and overwhelmingly dominated by cysts of Protoperidiniaceae, a family of exclusively heterotrophic cyst-producing dinoflagellates (21). Among the early Oligocene protoperidiniacean dinocysts, specimens closely resembling the modern *Selenopemphix antarctica* [here referred to as *S. cf. antarctica*] are recorded in low-to-moderate abundance (2 to 20%). *S. antarctica* is the dominant dinocyst taxon in the modern seasonal sea-ice zone of the Southern Ocean (supplementary text) (22, 23). At site U1356, the first appearance of dominant Protoperidiniaceae is accompanied by sedimentological indications of glacial activity, such as the deposition of outsized clasts (17), and increased sedimentary grain-size uniformity (Fig. 2). Furthermore, this assemblage shift is accompanied by an increase in the abundance and diversity of pyritized siliceous microfossils at site U1356, reflecting elevated primary productivity (supplementary text).

In order to determine whether the distinctive, low-diversity protoperidiniacean dinocyst assemblages occurred widely around Antarctica and whether the success of this plankton group was directly linked with glacial expansion in the earliest Oligocene, we also investigated dinocyst assemblages from three other sediment cores at sites positioned close to the Antarctic margin (Fig. 3). We used the first occurrence (FO) of *M. escutiana* as a marker for the correlative level of Oi1 (18). At Ocean Drilling Program (ODP) site 739 in Prydz Bay (Fig. 1), relatively diverse high-latitude assemblages with small Protoperidiniaceae in low abundance characterize the upper Eocene interval (fig. S4). These are replaced by dominant Protoperidiniaceae, including *S. cf. antarctica*, at the level correlative to Oi1, where biogenic silica (i.e., diatom) concentrations also significantly increase (fig. S4). A relatively complete Eocene-Oligocene succession was recovered at ODP site 696 in the Weddell Sea (Fig. 1). There, the early late Eocene interval is also characterized by relatively diverse high-latitude assemblages with abundant (>20%) small Protoperidiniaceae (fig. S5). At the EOT, the Protoperidiniaceae (including *S. cf. antarctica*) become dominant. At Cape Roberts Drilling Project site CRP-3 in the Ross Sea (Fig. 1), dinocysts are scarce and poorly preserved across the EOT interval (24). Further up-section (above 190 mbsf), however, well-preserved assemblages are present and include *M. escutiana*, implying an earliest Oligocene age, and dominant Protoperidiniaceae, similar to the assemblages recorded at the other circum-Antarctic sites (supplementary text).

To assess whether biotic turnover across the EOT was indeed restricted to the circum-Antarctic regions, we also considered time-equivalent lower Oligocene successions from ODP sites 1128 (Australian Bight), 1168 (west of Tasmania), and 1172 (East Tasman Plateau), which are located more distally from the Antarctic margin in the Southern Ocean. All early Oligocene assemblages at these sites are devoid of protoperidiniacean dinocysts (supplementary text). Instead, they are characterized by relatively diverse dinocyst assemblages that

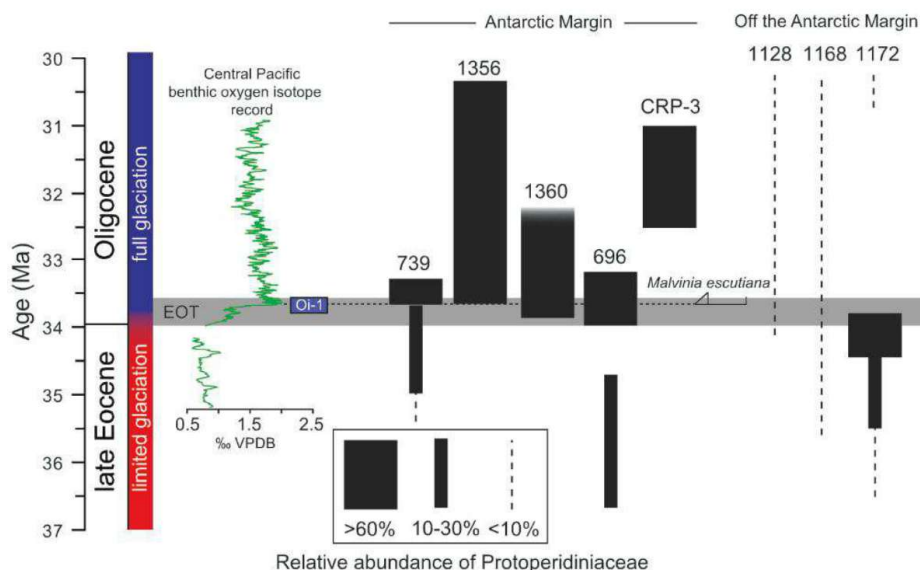


Fig. 3. Summary of the relative abundance of protoperidiniacean dinocysts at circum-Antarctic drill sites. The deep-sea oxygen isotope record is after (4), and Oi1 marks the final large oxygen isotope shift of the EOT (gray band). Large specimens of *S. cf. antarctica* and *M. escutiana* occur from Oi1 onward. If no bar is indicated, there is no sedimentary record for that time interval. Protoperidiniacean dinocysts abundant in the Upper Eocene of ODP site 1172 solely comprise *Brigantedinium* spp. and are likely related to oceanic upwelling conditions at this location (35) (supplementary materials).

were produced by autotrophic dinoflagellates (supplementary materials). Late Eocene assemblages at site 1172 nonetheless contain abundant protoperidiniacean dinocysts (Fig. 3); however, these protoperidiniacean taxa exclusively comprise the genus *Brigantedinium* and are interpreted to indicate strong oceanic upwelling (23). The absence of the typical Protoperidiniaceae at these sites illustrates that the appearance of near-modern Antarctic dinocyst assemblages at Oi1 was geographically restricted to areas close to the Antarctic margin (Fig. 3).

There are several possible explanations for the distinct shift in dinocyst assemblages across Oi1 at sites around the Antarctic margin. First, modern protoperidiniacean dinocyst taxa include cosmopolitan generalist heterotrophs that are abundant in a wide array of environments, such as deltaic (25) and open-ocean upwelling settings (26). Hence, the abrupt occurrence of protoperidiniacean dinocysts may have been connected with high primary productivity resulting from invigorated vertical mixing associated with intensified polar easterly wind fields after the emplacement of the Antarctic ice sheet (7). This mechanism would have primarily contributed to high abundance of heterotrophic Protoperidiniaceae in the modern-day domain of the Antarctic Circumpolar Current (22) and therefore fails to explain why Protoperidiniaceae became particularly abundant at Antarctic shelf sites. It also fails to explain why non-protoperidiniacean heterotrophic taxa that were already abundant in the Eocene Southern Ocean (16) did not proliferate, particularly at ODP site 1168. Second, a transient increase in nutrient availability in shelf waters caused by enhanced continental erosion during the Oi1 glacial expansion (27) could have contributed to high productivity and the successive appearance of the heterotrophic dinoflagellate communities around Antarctica. However, this scenario does not account for the dominance of protoperidiniacean communities during subsequent glacial-interglacial cycles after Oi1. These cycles would have likely been characterized by highly variable nutrient input to Antarctic shelf waters on orbital (20 to 400 thousand years) time scales, resulting in pulsed abundance distributions of protoperidiniaceans; we instead record stable protoperidiniacean-dominated assemblages throughout the early Oligocene.

Modern Antarctic phytoplankton communities are adapted to seasonal sea-ice conditions, either as plankton in the upper surface waters, ice-dwelling extremophiles occupying brine channels and pockets, or attached subice communities (8, 9). During the spring sea-ice melt, accumulated nutrients are released from the ice into the surrounding melt zone, resulting in a nutrient-rich shallow mixed layer that stimulates intensive and relatively short-lived blooms of primary producers (8–10). By analogy to the modern ecosystem, and considering an abrupt regional increase in siliceous sedimentation at Southern Ocean sites across Oi1 (28) and increased abundance and diversity of pyritized siliceous microfossils at site

U1356 (supplementary text), we conclude that seasonal blooms of phytoplankton initiated in the circum-Antarctic seas. This third scenario, involving an abrupt shift to high seasonal primary productivity associated with the development of seasonal sea ice, provides the most consistent explanation for the abundant appearance of protoperidiniacean dinocysts at Oi1. This mechanism is in agreement with numerical climate models simulations indicating that sea-ice formation along Antarctic margins may have followed full-scale Antarctic glaciation (7). Therefore, we propose that seasonal sea-ice formation was a critical factor in the early Oligocene reorganization of Antarctic plankton ecosystems.

Although the overall annual primary productivity in the modern sea-ice zone is generally low compared with other regions of the Southern Ocean (29), it does provide a highly concentrated and seasonal food source for grazers. This is illustrated by the pivotal role that high seasonal productivity associated with sea-ice conditions plays in the life-cycle of Antarctic krill (*Euphausia superba*) (30), a zooplankton group that sustains higher trophic level organisms in the Southern Ocean food web. The ecological response to Antarctic cryosphere development in the early Oligocene, as recorded by the rise to dominance of Protoperidiniaceae, may thus have been an important driver of niche differentiation, affecting many biotic groups. Indeed, molecular clock studies reveal important evolutionary events at higher trophic levels, such as the genetic divergence between baleen and toothed whales and the origination of extant penguins during the Eocene-Oligocene time interval (31, 32). We consequently infer that the pervasive environmental changes related to Antarctic glaciation in the early Oligocene, including sea-ice formation and a shift to intense seasonal primary productivity patterns, led to the concurrent rise of new plankton communities in Antarctic shelf waters and set the stage for major evolutionary turnover in metazoans.

References and Notes

1. Z. Liu *et al.*, *Science* **323**, 1187 (2009).
2. S. M. Bohaty, J. C. Zachos, M. L. Delaney, *Earth Planet. Sci. Lett.* **317–318**, 251 (2012).
3. H. K. Coxall, P. A. Wilson, H. Pälike, C. H. Lear, J. Backman, *Nature* **433**, 53 (2005).
4. K. G. Miller, J. D. Wright, R. G. Fairbanks, *J. Geophys. Res.* **96**, 6829 (1991).
5. J. C. Zachos, J. R. Breza, S. W. Wise Jr., *Geology* **20**, 569 (1992).
6. W. U. Ehrmann, A. Mackensen, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **93**, 85 (1992).
7. R. DeConto, D. Pollard, D. M. Harwood, *Paleoceanography* **22**, PA1350 (2007).
8. K. R. Arrigo *et al.*, *Science* **283**, 365 (1999).
9. D. N. Thomas, G. S. Dieckmann, *Science* **295**, 641 (2002).
10. M. P. Lizotte, *Am. Zool.* **41**, 57 (2001).
11. L. K. Armand, A. Leventer, in *Sea Ice*, D. N. Thomas, G. S. Dieckmann, Eds. (Wiley-Blackwell, Oxford, 2010), chap. 13.
12. A. Leventer, *Antarct. Res. Ser.* **73**, 121 (1998).
13. E. E. Kopczynska, N. Savoye, F. Dehairs, D. Cardinal, M. Elskens, *Polar Biol.* **31**, 77 (2007).
14. E. Balech, Dinoflagellates, in *Antarctic Map Folio Series* (Folio 10, American Geophysical Society, New York, 1968).
15. A. Sluijs, J. Pross, H. Brinkhuis, *Earth Sci. Rev.* **68**, 281 (2005).
16. P. K. Bijl *et al.*, *Paleoceanography* **26**, PA1202 (2011).
17. C. Escutia *et al.*, Proceedings of the Integrated Ocean Drilling Program Expedition 318, in *Integrated Ocean Drilling Program Management International* (Ocean Drilling Program, Tokyo, 2011), vol. 318, 10.2204/ioldp.proc.318.2011.
18. A. J. P. Houben, P. K. Bijl, G. R. Gierstein, A. Sluijs, H. Brinkhuis, *Rev. Palaeobot. Palynol.* **165**, 175 (2011).
19. L. Tauxe *et al.*, *Paleoceanography* **27**, PA2214 (2012).
20. Information on materials and methods is available in the supplementary materials on Science Online.
21. D. M. Jacobson, D. M. Anderson, *J. Physiol.* **22**, 249 (1986).
22. F. Marret, A. De Vernal, *Mar. Micropaleontol.* **29**, 367 (1997).
23. R. Harland, C. J. Pudsey, *Mar. Micropaleontol.* **37**, 77 (1999).
24. M. J. Hannah, J. H. Wrenn, G. J. Wilson, *Terra Antarctica* **8**, 383 (2001).
25. I. Bouimatarhan, F. Marret, L. Dupont, K. A. F. Zonneveld, *Mar. Micropaleontol.* **71**, 113 (2009).
26. G. Reichart, H. Brinkhuis, *Mar. Micropaleontol.* **49**, 303 (2003).
27. H. D. Scher, S. M. Bohaty, J. C. Zachos, M. L. Delaney, *Geology* **39**, 383 (2011).
28. J. Barron, J. Baldauf, in *Productivity of the Ocean: Present and Past*, W. H. Berger, V. S. Smetacek, G. Wefer, Eds. (Wiley, New York, 1989), pp. 341–354.
29. K. R. Arrigo, G. L. van Dijken, S. Bushinsky, *J. Geophys. Res.* **113**, C08004 (2008).
30. A. S. Brierley *et al.*, *Science* **295**, 1890 (2002).
31. M. E. Steeman *et al.*, *Syst. Biol.* **58**, 573 (2009).
32. A. J. Baker, S. L. Pereira, O. P. Haddad, K. A. Edge, *Proc. Biol. Sci.* **273**, 11 (2006).
33. D. S. Wilson *et al.*, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **335–336**, 24 (2012).
34. L. A. Lawler, L. C. Gahagan, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **198**, 11 (2003).
35. C. E. Stickley *et al.*, *Paleoceanography* **19**, 10.1029 (2004).

Acknowledgments: This research used samples from the ODP and the IODP. The ODP was sponsored by the NSF and participating countries under management of Joint Oceanographic Institutions (JOI) Incorporated. A.J.P.H. and H.B. thank Statoil for funding. P.K.B., F.S., and H.B. thank the Netherlands Organisation for Scientific Research (NWO) (grant 86610110) and the LPP Foundation for funding. J.P. and U.R. acknowledge support through the German Research Foundation (grants PR 651/10 and RO 1113/6). J.P. thanks the Biodiversity and Climate Research Center within the Hessian Initiative for Scientific and Economic Excellence (LOEWE) for support. S.P. acknowledges financial support from a Post-Expedition Activity award administered by the U.S. Science Support Program and research support from NSF award ANT-1245283. T.v.d.F. acknowledges the Natural Environmental Research Council (UK) for a cruise participation grant (NE/H025162/1) and the European Commission for a Marie Curie Reintegration grant (IRG 230828). S.M.B. and T.v.d.F. further acknowledge UK IODP funding (grant NE/I006257/1). S.M.B. acknowledges the Natural Environmental Research Council (UK) for a cruise participation grant (NE/H020098/1) and postcruise research support (NE/J019801/1). L.T. acknowledges support from NSF grants OCE 1058858 and OCE 1054497. A.S. thanks the European Research Council for ERC Starting Grant 259627. C.E. thanks the Spanish Ministry for Science and Innovation (grant CTM2011-2079). The reported data are available in the supplementary materials.

Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6130/341/DC1

Materials and Methods

Supplementary Text

Figs. S1 to S6

Tables S1 to S3

Plate S1

References (36–92)

Data

23 April 2012; accepted 15 February 2013

10.1126/science.1223646

Global Charcoal Mobilization from Soils via Dissolution and Riverine Transport to the Oceans

Rudolf Jaffé,^{1*†} Yan Ding,¹ Jutta Niggemann,² Anssi V. Vähätalo,^{3,4} Aron Stubbins,⁵ Robert G. M. Spencer,⁶ John Campbell,⁷ Thorsten Dittmar^{2*†}

Global biomass burning generates 40 million to 250 million tons of charcoal every year, part of which is preserved for millennia in soils and sediments. We have quantified dissolution products of charcoal in a wide range of rivers worldwide and show that globally, a major portion of the annual charcoal production is lost from soils via dissolution and subsequent transport to the ocean. The global flux of soluble charcoal accounts to 26.5 ± 1.8 million tons per year, which is $\sim 10\%$ of the global riverine flux of dissolved organic carbon (DOC). We suggest that the mobilization of charcoal and DOC out of soils is mechanistically coupled. This study closes a major gap in the global charcoal budget and provides critical information in the context of geoengineering.

Charcoal, or black carbon (BC), is a molecularly diverse organic product of incomplete combustion of biomass and fossil fuels (1–3). It is ubiquitous in the environment, where it slowly decomposes (4), but part of it is preserved for thousands of years (5). Global biomass burning generates an estimated 40 to 250 mega-metric tons (MMT) of BC per year (6) (MMT = 10^{12} grams), and 5 to 40% of total soil organic carbon (OC) is estimated to be BC (7–10). The global BC stock in sediments, soils, and waters combined is 300 to 500 giga-metric tons of carbon (11). BC has been considered as extraordinarily stable in the environment. However,

current production rates exceed estimates of decomposition rates. According to this mismatch, there should be more BC than total OC in soils (12, 13). This paradox indicates that a relatively labile BC pool must exist, allowing for considerable losses from soils. Measurements of BC age in boreal forest soils of ~ 652 years (14) agree with reported BC degradation rates on centennial time scales (11, 15, 16), suggesting that microbial degradation and erosion of BC may be more substantial than previously believed. A meta-analysis of the current knowledge on BC degradation in soils (4) indicates turnover times of less than 100 years. Alternatively, multiple pools

of BC were suggested in the same study (4), with about half of the BC turning over in 3 years, whereas the other half may persist in soils over millennia. This range of reactivity is probably a function of charring temperature and source material, but also, environmental factors such as soil properties and fauna play a role in BC decomposition (4, 17, 18). In addition to complete oxidation into CO_2 and particle erosion (19), BC can also be mobilized from soils in dissolved form as dissolved BC (DBC) (11, 20–23). A recent study showed that even decades after major slash and burn, DBC was efficiently mobilized in large quantities from the soils of a former tropical forest biome in Brazil (21). The fact that $>2\%$ of the marine dissolved OC (DOC) pool has a heat-induced molecular signature (24) also points toward considerable fluxes of DBC from land to ocean. If BC dissolution in soils is an important

¹Southeast Environmental Research Center (SERC), and Department of Chemistry and Biochemistry, Florida International University (FIU), Miami, FL 33199, USA. ²Max Planck Research Group for Marine Geochemistry, Institute for Chemistry and Biology of the Marine Environment, University of Oldenburg, 29129 Oldenburg, Germany. ³Department of Environmental Science, University of Helsinki, 00014 Helsinki, Finland. ⁴Department of Biological and Environmental Sciences, University of Jyväskylä, 40500 Jyväskylä, Finland. ⁵Skidaway Institute of Oceanography, 10 Ocean Science Circle, Savannah, GA 31411, USA. ⁶Woods Hole Research Center, 149 Woods Hole Road, Falmouth, MA 02540, USA. ⁷U.S. Department of Agriculture, Forest Service, Northern Research Station, Durham, NH 03824, USA.

*Corresponding author. E-mail: jaffer@fiu.edu (R.J.); tdittmar@mpi-bremen.de (T.D.)

†These authors contributed equally to this work.

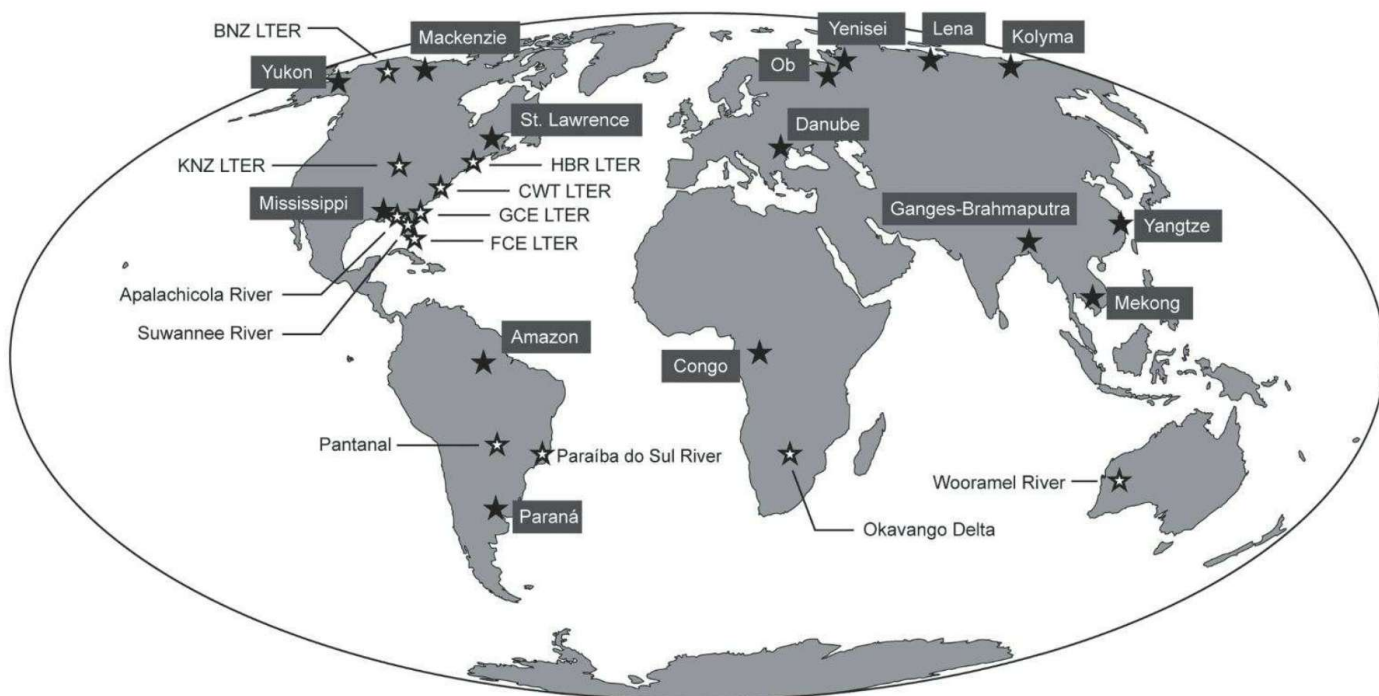


Fig. 1. Map of global freshwater DBC sampling sites. Black stars indicate major world rivers, and white stars indicate all other sites, including minor to intermediate rivers and wetland-associated streams, including Long-Term Ecological Research (LTER) sites. BNZ, Bonanza Creek; KNZ, Konza Prairie; HBR, Hubbard Brook; CWT, Coweeta; GCE, Georgia Coastal Ecosystems; FCE, Florida Coast Everglades.

translocation process in terrestrial systems, the transfer of this material to marine environments may be critical in establishing global mass balances for BC. However, few data on DBC loads are available for rivers (21, 23), and this quantitative information is too limited to reliably estimate land-ocean fluxes on a global scale.

The objective of this study was to provide an estimate of the global flux of DBC from land to ocean through rivers. For this purpose, we determined the concentration of DBC in rivers globally (Fig. 1) across a wide range of sizes, from major rivers to headwater streams and wetland watersheds, and geographically from the Arctic to the southern midlatitudes.

DBC was determined on a molecular level as the sum of polycyclic aromatic carbon (25). This polycyclic aromatic fraction forms at the highest temperatures (7) and is among the most stable components of charred biomass. It is also the fraction of BC that is found dissolved throughout the deep ocean (24). The limitation of our analytical window to polycyclic aromatic carbon implies that the global DBC flux calculations reported here are conservative underestimates and comprise a lower limit of the actual fluxes. We studied 174 freshwater samples in order to assess the average and variability of DBC and DOC concentrations on a globally representative scale. Sampling was not necessarily intended to resolve seasonal or site-specific features, such as climate, elevation, or vegetation. Replicate samples from the same locations and season were averaged so as to avoid domination of the statistics by only a few sampling locations, thus reducing the overall data set to 109 data points. Each data point represents a distinct main stem, tributary, wetland system, or season from 27 different river systems and wetlands. These samples include 15 of the largest rivers worldwide, such as the Amazon, Congo, and major Arctic rivers. We appended the data set with results from previous case studies (20–22).

The DBC concentrations varied strongly among the freshwater systems, ranging from 1.94×10^{-3} to $2.77 \text{ mg carbon liter}^{-1}$ (average $\pm$ SD of $0.48 \pm 0.55 \text{ mg carbon liter}^{-1}$). DBC comprised from 0.1 to 17.5% of DOC (average $\pm$ SD of $7.1 \pm 3.6\%$). Despite this apparent variability, DBC and DOC concentrations were highly correlated [correlation coefficient (r^2) = 0.88, n = 109 data points, P < 0.0001]. Recent case studies that examined the relationship between DOC and DBC (20–22), as well as soil BC and soil organic matter (10, 26), are consistent with our observation. Because our DOC and DBC concentrations were not normally distributed, we grouped the samples according to their DOC concentrations into 15 groups for further statistical analysis (Fig. 2). The slope of the resulting regression indicates that the global DOC pool contains 10.6% DBC ($\pm 0.7\%$, on a 95% confidence level, n = 15 DOC concentration groups). The intercept of the regression was not significantly different from zero. If the original ungrouped data are used for the regression, the resulting slope and intercept are

statistically not different from the grouped data, but the estimates for the error margins (confidence intervals) are lower because of the higher number of data points (n = 109 data points). The major world river samples followed the regression of the whole data set, suggesting that natural integration occurs in big watersheds.

We took advantage of this highly significant relationship between DBC and DOC concentrations to convert published data for global river loads of DOC into DBC loads. The estimated annual DBC flux from land to ocean is about $26.5 \pm 1.8 \text{ MMT}$ of carbon per year, based on a flux of riverine DOC of 250 MMT of carbon per year (27). The global DOC flux contains considerable margins of uncertainty. As refinements of global DOC flux estimates become available, updates on global DBC fluxes can be easily made on the basis of the observed relationship between DOC and DBC.

Although a wide range of variability among individual rivers was observed as expected, the global linear relationship between DOC and DBC concentrations appears enigmatic on a first view. It suggests that the diagenetic processes driving the release of DOC and DBC from soils are related. The close coupling between DBC and DOC fluxes indicates that the release and subsequent sorption and desorption processes of DBC are a complex function of environmental factors and soil properties, just as for bulk DOC. The aging of BC in soils seems critical in facilitating its transfer to the aqueous phase (21, 22, 28). During

aging, the condensed aromatic ring structures of charcoal are functionalized over time with hydroxyl and carboxylic groups on their periphery (11, 29), conferring them not only some limited aqueous solubility but also binding capacities to mineral surfaces in soil (10, 11). As an alternative explanation, DOC could be directly involved in the physical translocation process of soil BC to DBC. As for hydrophobic organic pollutants, the presence of dissolved organic matter may serve as a mobilizing agent through hydrophobic interactions.

According to our data, it is clear that DBC is globally ubiquitous in freshwater environments. Approximately 26.5 MMT carbon of DBC enter the oceans every year, which is in the same order of magnitude as the estimated range of the annual production of BC from vegetation fires (40 to $250 \text{ MMT carbon year}^{-1}$ of BC) (6). Although other soil processes—such as microbial oxidation, sorption of DBC on aquifer materials, erosion, and reburning—also may contribute to the loss of soil BC, our study shows that DOC is an important environmental intermediate for both transfer and storage of BC. With continued anthropogenic perturbations to watersheds (such as slash-and-burn agriculture) and global climate change impacts (such as permafrost thaw) leading to an increase in terrestrial DOC export from land to the oceans, the flux of DBC is expected to increment equivalently. Bio-char applications to soils have been proposed as an effective means of carbon sequestration (30). This activity may further

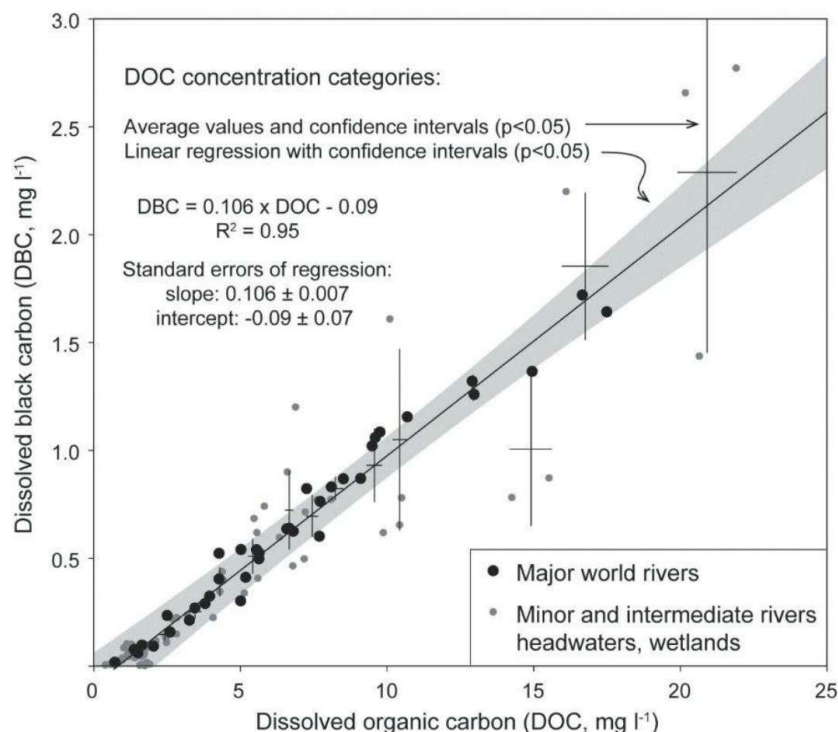


Fig. 2. DBC versus DOC concentrations of global rivers. The regression parameters are for the average values of 15 DOC concentration groups (crosses). Raw data regression yields the same slope and intercept, but the confidence intervals are smaller because of the larger number of samples.

enhance the translocation and export of DBC to marine systems. The environmental consequences of this are presently unknown but may be reflected in the reduction of DOC bioavailability and associated effects on microbial loop dynamics and aquatic food webs. Our data suggest that we apply our existing knowledge on DOC production, storage, and movement in soils to ensure that biochar applications are implemented sustainably and managed in ways to minimize riverine DBC fluxes.

References and Notes

1. J. A. Baldock, R. J. Smernik, *Org. Geochem.* **33**, 1093 (2002).
2. M. P. W. Schneider, M. Hilf, U. F. Vogt, M. W. I. Schmidt, *Org. Geochem.* **41**, 1082 (2010).
3. E. D. Goldberg, *Black Carbon in the Environment: Properties and Distribution* (Wiley, New York, 1985).
4. N. Singh, S. Abiven, M. S. Torn, M. W. I. Schmidt, *Biogeochemistry* **9**, 2847 (2012).
5. C. A. Masiello, E. R. M. Druffel, *Science* **280**, 1911 (1998).
6. T. A. J. Kuhlbusch, P. J. Crutzen, in *Biomass Burning and Global Change*, J. S. Levine Ed. (MIT Press, Cambridge, MA 1996), chap. 16.
7. K. Hammes et al., *Global Biogeochem. Cycles* **21**, GB3016 (2007).
8. J. O. Skjemstad, D. C. Reicosky, A. R. Wilts, J. A. McGowan, *Soil Sci. Soc. Am. J.* **66**, 1249 (2002).
9. C. A. Masiello, E. R. M. Druffel, *Geophys. Res. Lett.* **30**, 1185 (2003).
10. D. Cusack, O. A. Chadwick, W. C. Hockaday, P. M. Vitousek, *Global Biogeochem. Cycles* **26**, GB2019 (2012).
11. W. C. Hockaday, A. M. Grannas, S. Kim, P. G. Hatcher, *Geochim. Cosmochim. Acta* **71**, 3432 (2007).
12. C. A. Masiello, *Mar. Chem.* **92**, 201 (2004).
13. M. W. I. Schmidt, *Nature* **427**, 305 (2004).
14. M. Ohlson, B. Dahlberg, T. Okland, K. J. Brown, R. Halvorsen, *Nat. Geosci.* **2**, 692 (2009).
15. C. I. Czimczik, C. M. Preston, M. W. I. Schmidt, E. D. Schulze, *Global Biogeochem. Cycles* **17**, GB001956 (2003).
16. M. I. Bird, C. Moyo, E. M. Veenendaal, J. Lloyd, P. Frost, *Global Biogeochem. Cycles* **13**, 923 (1999).
17. C. I. Czimczik, C. A. Masiello, *Global Biogeochem. Cycles* **21**, GB003005 (2007).
18. B. T. Nguyen, J. Lehmann, W. C. Hockaday, S. Joseph, C. A. Masiello, *Environ. Sci. Technol.* **44**, 3324 (2010).
19. S. Mitra, T. S. Bianchi, B. A. McKee, M. Sutula, *Environ. Sci. Technol.* **36**, 2296 (2002).
20. T. Dittmar, J. Paeng, T. M. Gihring, I. G. N. A. Suryaputra, M. Huettel, *Limnol. Oceanogr.* **57**, 1171 (2012).
21. T. Dittmar et al., *Nat. Geosci.* **5**, 618 (2012).
22. Y. Ding, Y. Yamashita, W. K. Dodds, R. Jaffé, *Chemosphere* **90**, 2557 (2013).
23. A. Mannino, H. R. Harvey, *Limnol. Oceanogr.* **49**, 735 (2004).
24. T. Dittmar, J. Paeng, *Nat. Geosci.* **2**, 175 (2009).
25. T. Dittmar, *Org. Geochem.* **39**, 396 (2008).
26. B. Glaser, W. Amelung, *Global Biogeochem. Cycles* **17**, GB002019 (2003).
27. J. I. Hedges, R. G. Keil, R. Benner, *Org. Geochem.* **27**, 195 (1997).
28. S. Abiven, P. Hengartner, M. P. W. Schneider, N. Singh, M. W. I. Schmidt, *Soil Biol. Biochem.* **43**, 1615 (2011).
29. T. Dittmar, B. P. Koch, *Mar. Chem.* **102**, 208 (2006).
30. J. Lehmann, J. Gaunt, M. Rondon, *Mitig. Adapt. Strat. Gl.* **11**, 403 (2006).

Acknowledgments: R.J. and Y.D. thank the NSF [grant DBI-0620409; Florida Coast Everglades–LTER program], the SERC Barley Chair, and the FIU–University Graduate School for financial support. The Academy of Finland funded A.V.V. and the collection of samples from large rivers. R.G.M.S. was supported by NSF grants (ETBC-0851101, DEB-1145932, ANT-1203885, and OPP-1107774). A.S. was supported by the NSF (DEB-1146161) and a Fellowship from the Hanse Institute for Advanced Studies (HWK, Delmenhorst, Germany). R. Max Holmes, James W. McClelland, and the rest of the Arctic Great Rivers Observatory (www.arcticgreatrivers.org) team are thanked for providing Arctic river samples (NSF OPP-0732522, OPP-0732821, and OPP-1107774). We thank Y. Yamashita, J. Jones (Bonanza Creek–LTER), W. Dodds (Konza Prairie–LTER), C. Craft (Georgia Coastal Ecosystems–LTER), N. Jaffé, C. Nunes, K. Cawley, P. Wolski, R. Price, C. Rezende, E. M. Paolucci, E. Musibono, A. Shantz, Y. Gélinas, S. R. Khan, Q. Huang, W. Schneider, E. Petrescu and H. E. Reader for providing samples and for field support and M. Friebe and I. Ulber for analytical support. This is SERC Contribution 593. Original data will be available at http://fclter.fiu.edu/data/public_data_access/Rjaffe_Global_DissolvedBlack_Corg.xls.

Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6130/345/DC1
Materials and Methods
Supplementary Text
References (31, 32)

15 October 2012; accepted 8 February 2013
10.1126/science.1231476

Resilience and Recovery of Overexploited Marine Populations

Philipp Neubauer,^{1*} Olaf P. Jensen,¹ Jeffrey A. Hutchings,^{2,3} Julia K. Baum⁴

Recovery of overexploited marine populations has been slow, and most remain below target biomass levels. A key question is whether this is due to insufficient reductions in harvest rates or the erosion of population resilience. Using a global meta-analysis of overfished stocks, we find that resilience of those stocks subjected to moderate levels of overfishing is enhanced, not compromised, offering the possibility of swift recovery. However, prolonged intense overexploitation, especially for collapsed stocks, not only delays rebuilding but also substantially increases the uncertainty in recovery times, despite predictable influences of fishing and life history. Timely and decisive reductions in harvest rates could mitigate this uncertainty. Instead, current harvest and low biomass levels render recovery improbable for the majority of the world's depleted stocks.

Recovery of overexploited marine populations would be a “win-win” outcome for fisheries and conservation, easing pressure on wild populations and associated ecosystems (1–3), and ultimately enhancing catches, revenues, and food security (4–6). Recognizing

the global importance of recovery, the United Nations (UN) 2002 World Summit on Sustainable Development proposed that global fisheries be rebuilt to maximum sustainable yield (MSY) levels by 2015 (5, 7). Echoing this call, several countries, including Australia and the United States, mandated rebuilding in their fisheries legislation. In Europe, a proposed new Common Fisheries Policy (CFP) aims to rebuild fisheries to MSY levels by 2020 (8). Although exploitation rates have been reduced and population declines halted in some regions (9, 10), stock biomass remains below that of maximum sustainable yield (B_{MSY}) for the majority of commercially exploited fishes (6, 10, 11). By 2010, only ~1% of global

stocks requiring rebuilding had been successfully rebuilt (9), suggesting that the UN's 2015 recovery target will not be met (5). Here, we empirically examine the time frames needed to recover the world's depleted stocks.

Fisheries operate in complex systems, and the factors driving recovery remain uncertain. Simple theoretical models predict that most overfished stocks should recover to B_{MSY} in less than a decade if fishing mortality is sufficiently reduced (12). The slow recovery of some populations, however, has led to increased investigation of their resilience to overfishing (13, 14). Allee effects (13, 15), reduced productivity attributable to adverse environmental conditions (16, 17), and the evolution of life-history traits caused by high fishing mortality (18, 19) have been proposed as causes of impaired recoveries. Although such effects may be identifiable retrospectively [e.g., (20)], it remains unclear whether recoveries are predictable, given these potentially adverse effects of long-term exploitation and altered ecosystem states.

To gain insight into the recovery process, we investigated the importance of fishing mortality relative to life history, exploitation history, and other hypothesized drivers of recovery for 153 marine fish and invertebrate stocks for which stock assessments indicated that population biomass had declined below half of B_{MSY} (table S1) (11, 21). We modeled recovery times—the number of years between depletion ($B < 0.5 B_{MSY}$) and recovery to B_{MSY} —for 184 depletion events, using a Bayesian regression model based on

¹Institute of Marine and Coastal Sciences, Rutgers University, New Brunswick, NJ 08901, USA. ²Department of Biology, Dalhousie University, 1355 Oxford Street, Halifax, NS B3H 4R2, Canada. ³Centre For Ecological and Evolutionary Synthesis, Department of Biosciences, University of Oslo, Oslo, Norway. ⁴Department of Biology, University of Victoria, Post Office Box 1700 STN CSC, Victoria, British Columbia V8W 2Y2, Canada.

*Corresponding author. E-mail: neubauer.phil@gmail.com

stochastic population dynamics. Our models incorporate survival analysis methods to accommodate the “censored” nature of the data for stocks whose recovery is incomplete (22). From these models, we predict expected recovery times, quantify their uncertainty, and determine conditions under which population resilience is compromised.

Recovery is a multifaceted process. Our models reveal that it is driven not only by the minimum relative biomass to which stocks are depleted (B/B_{MSY}), their relative fishing mortality during recovery (F/F_{MSY}), and their intrinsic rate of increase (r), but also by their exploitation history—the interplay between how long and how hard stocks are fished before their depletion (Fig. 1). On their own, exploitation time (the time between the development year of a fishery and its year of depletion) and historic fishing intensity (the long-term average relative fishing mortality during this time) are positive influences, shortening recovery time (Fig. 1). The interaction of these two variables (exploitation history) is, however, negative. Although particularly intense exploitation histories (e.g., $6F_{MSY}$) may thus slow recoveries (Fig. 2, A and C), moderate levels of overexploitation before depletion (e.g., $2F_{MSY}$) appear to enhance the resilience of stocks to such harvest regimes, resulting in shorter rebuilding times once they are depleted (all else being equal) (Figs. 1A, 2, A and C, and 3A).

Our results are consistent with the hypothesis that moderate overexploitation can generate life-history changes, which increase r to a value greater than that of a population that has not adapted to overfishing. Plastic and evolutionary changes in maturation schedules appear to compensate for initial losses in spawning output caused by selective removal of relatively large, fecund individuals (22, 23) and thus increase resilience to overfishing (22). These changes are, however, relatively slow and likely scale with applied fishing pressure and duration (22–24). Stocks that are depleted over a relatively short time (e.g., <30 years) through moderate overfishing thus exhibit substantially slower and less predictable recovery rates than those that experienced a long history of overfishing (e.g., >50 years) at the same moderate rate before depletion (Fig. 2A). The former may occur for depletions that are caused at least partly by adverse environmental regimes and/or for overfished populations that had insufficient time to adapt to fishing regimes.

Although our analysis also provides some evidence that intense long-term overfishing eventually impedes resilience, such negative effects are predicted to manifest only at extremely high and sustained fishing mortalities, which occur rarely in our data (Fig. 3A and fig. S1). This empirical result is consistent with recent theoretical studies suggesting that maladaptive effects of fishing are likely slow to manifest and may have limited negative effect on recovery to B_{MSY} levels (22). Nonetheless, the altered phenotypic and genotypic composition resulting from continued over-

exploitation might be undesirable for reasons other than recovery per se, such as increased variability in biomass trends and decreased resilience to adverse environmental conditions that may compromise long-term productivity of these populations.

We find that recovery is generally predictable and achievable within a decade for stocks with average exploitation histories (i.e., historic fishing intensity $\sim 2.3F_{MSY}$ and exploitation times >30 years) and intrinsic rates of increase ($r = 0.43$) if fishing mortality is rapidly reduced below F_{MSY} once stocks fall below $0.5B_{MSY}$ and collapse ($B < 0.2B_{MSY}$) is avoided (Figs. 2, B and D, 3B, and fig. S3). For instance, reductions in F below F_{MSY} seem to have reversed the steady

and highly publicized trend toward population collapse of Eastern Atlantic bluefin tuna (*Thunnus thynnus*), and this stock is projected to recover within this time frame under fishing reductions to $F \leq F_{MSY}$ (Fig. 4A), a prediction supported by its most recent stock assessment (25). In general, however, uncertainty about rebuilding times is considerable if fishing rates are reduced merely to F_{MSY} once a stock is depleted. Median recovery times increase only from 5 to 8 years with a change in F from zero to F_{MSY} , but uncertainty in these times (25th and 75th percentiles of recovery times) increases from 3 to 10 up to 5 to 27 years (Fig. 2B, light green).

Not only does uncertainty about recovery time increase exponentially with applied fishing pres-

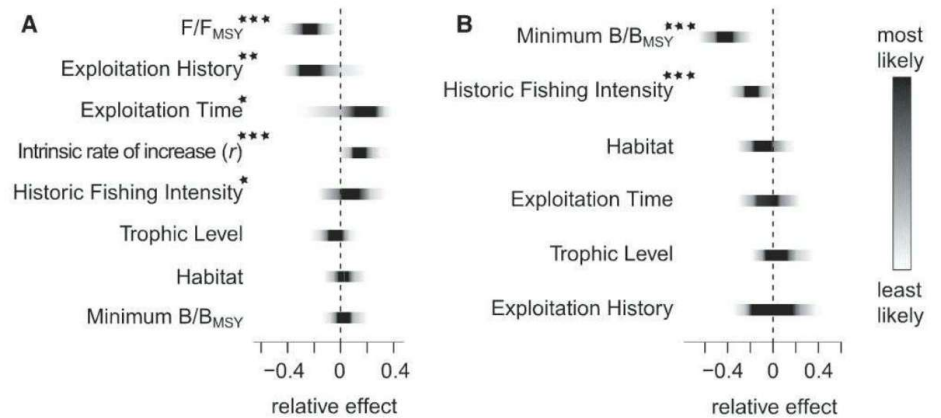


Fig. 1. Scaled posterior distributions of the effect of regression covariates on two components of recovery time. (A) Recovery rate and (B) relative distance from the recovery target (relative to time-series variance) (21). Dashed lines mark (A) $\beta = 0$ and (B) $\gamma = 0$, where covariates have no effect; one, two, and three stars denote 80, 90, and 99% probabilities that the effect is different from 0, respectively. In (A) a negative effect of a covariate (indicative of a lower recovery rate) delays recovery, whereas in (B) a negative effect of a covariate (indicative of a shorter relative distance) shortens recovery. Habitat is a categorical variable for pelagic and demersal stocks, coded to show the effect for pelagic stocks.

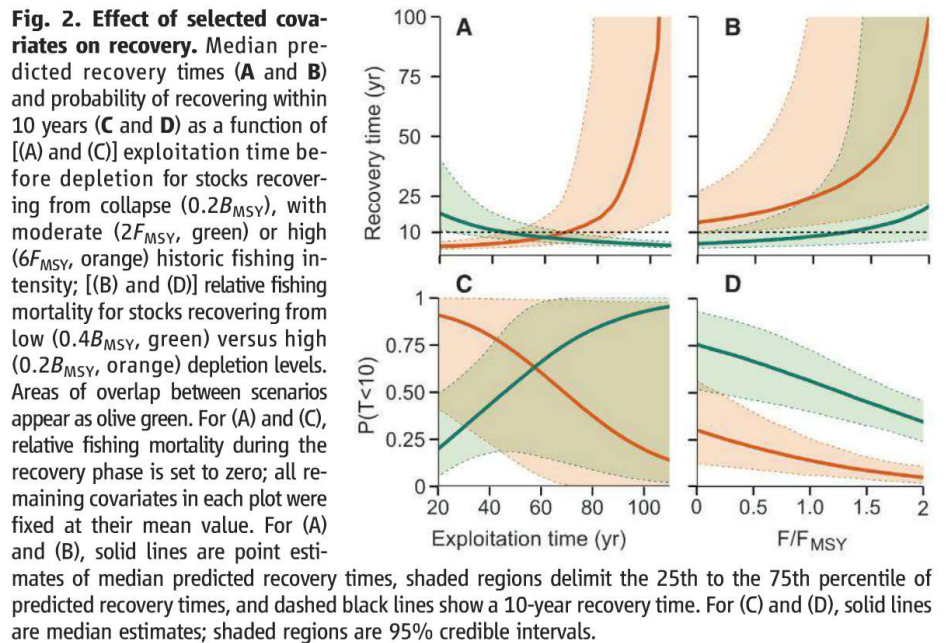


Fig. 2. Effect of selected covariates on recovery. Median predicted recovery times (A and B) and probability of recovering within 10 years (C and D) as a function of [(A) and (C)] exploitation time before depletion for stocks recovering from collapse ($0.2B_{MSY}$), with moderate ($2F_{MSY}$, green) or high ($6F_{MSY}$, orange) historic fishing intensity; [(B) and (D)] relative fishing mortality for stocks recovering from low ($0.4B_{MSY}$, green) versus high ($0.2B_{MSY}$, orange) depletion levels. Areas of overlap between scenarios appear as olive green. For (A) and (C), relative fishing mortality during the recovery phase is set to zero; all remaining covariates in each plot were fixed at their mean value. For (A) and (B), solid lines are point estimates of median predicted recovery times, shaded regions delimit the 25th to the 75th percentile of predicted recovery times, and dashed black lines show a 10-year recovery time. For (C) and (D), solid lines are median estimates; shaded regions are 95% credible intervals.

sure during the recovery phase but also recovery times are prolonged and less predictable for collapsed stocks (Fig. 2, B and E). For some collapsed Atlantic cod (*Gadus morhua*) stocks, for example, although median predicted rebuilding times are ~10 years, there is substantial uncertainty about these rebuilding times (Fig. 4B). When stocks are driven to severely low abundances, recovery can take decades and recovery projections become uncertain. Georges Bank/Gulf of Maine Atlantic halibut (*Hippoglossus hippoglossus*)—a stock that had been overfished and at low biomass levels ($B \sim 0.01B_{MSY}$) for about a century before rebuilding began—exemplifies such slow and uncertain recoveries (Fig. 4C). Delays in recovery also are pronounced for stocks with low intrinsic rates of increase (e.g., $r < 0.2$). Species with such “slow” life histories constitute a group that is at particular risk of long-term depletion, especially if the fishing mortality rate is not sufficiently reduced (Fig. 3B and fig. S2).

The stochastic nature of the recovery process means that with longer recovery times comes much greater uncertainty. Patterns in productivity attributable to adaptive processes, trophic interactions, or environmental regimes can dictate the impact of a particular fishing regime and are thus important unknowns in projected recovery times (20, 26–29). For instance, when overfishing continues after depletion, cumulative recovery probabilities for individual stocks still increase over

time (Fig. 4). Thus, despite a marked increase in predicted recovery times and their uncertainty (especially for collapsed stocks) (Fig. 2B), median predicted recovery times remain finite even at fishing mortalities as high as $2F_{MSY}$, which, in theory, should not result in recovery. Recovery in these cases might well depend on the adaptive potential of each stock and/or the occurrence of conditions favorable to high recruitment, neither of which can, in general, be predicted with much certainty.

Our analysis suggests that the majority of stocks are resilient to moderate overfishing and have a good chance of recovering within 10 years if fishing pressure is reduced rapidly and substantially once a stock has been determined to be depleted ($B \approx 0.5 \times B_{MSY}$). For many currently depleted stocks, however, rebuilding efforts have been slow to be enacted, and continued overfishing has led to their collapse. At present, a third remain collapsed. Even if fishing mortality rates were reduced to F_{MSY} , recovery would likely take several decades for many of these stocks (Fig. 3B). Regardless of their depletion level, at current fishing mortality rates, recovery to B_{MSY} remains a distant target for the majority of stocks that are now depleted ($n = 62$ stocks in our analysis). Only 23% of these stocks are fished below F_{MSY} , and only 10% are fished below $0.5F_{MSY}$. Recent evidence that production from wild-capture fisheries around the world could be increased were

these stocks to be rebuilt (5, 6) should provide an economic incentive to implement policy and management decisions that accelerate and expand rebuilding efforts.

References and Notes

1. A. D. M. Smith *et al.*, *Science* **333**, 1147 (2011).
2. C. J. Walters, V. Christensen, S. J. Martell, J. F. Kitchell, *ICES J. Mar. Sci.* **62**, 558 (2005).
3. P. M. Cury *et al.*, *Science* **334**, 1703 (2011).
4. R. Q. Grafton, T. Kompas, R. W. Hilborn, *Science* **318**, 1601 (2007).
5. Y. Ye *et al.*, *Fish Fisheries* (2012); 10.1111/j.1467-2979.2012.00460.x
6. C. Costello *et al.*, *Science* **338**, 517 (2012).
7. United Nations, Report of the World Summit on Sustainable Development, Johannesburg, South Africa, 26 August to 4 September 2002; www.un.org/ga/search/view_doc.asp?symbol=A/CONF.199/20&Lang=E
8. European Commission (2011), Europa, CFP Reform: Maximum Sustainable Yield; http://ec.europa.eu/fisheries/reform/docs/msy_en.pdf
9. S. A. Murawski, *ICES J. Mar. Sci.* **67**, 1830 (2010).
10. B. Worm *et al.*, *Science* **325**, 578 (2009).
11. D. Ricard, C. Minto, O. P. Jensen, J. K. Baum, *Fish Fish.* **13**, 380 (2012).
12. C. Safina, A. A. Rosenberg, R. A. Myers, T. J. I. Quinn 2nd, J. S. Collie, *Science* **309**, 707 (2005).
13. D. Keith, J. Hutchings, R. Hilborn, *Can. J. Fish. Aquat. Sci.* **69**, 1150 (2012).
14. J. A. Hutchings, J. D. Reynolds, *Bioscience* **54**, 297 (2004).
15. C. Walters, J. F. Kitchell, *Can. J. Fish. Aquat. Sci.* **58**, 39 (2001).
16. Y. Jiao, *Rev. Fish Biol. Fish.* **19**, 177 (2009).
17. J. S. Choi, K. T. Frank, W. C. Leggett, K. Drinkwater, *Can. J. Fish. Aquat. Sci.* **61**, 505 (2004).
18. M. R. Walsh, S. B. Munch, S. Chiba, D. O. Conover, *Ecol. Lett.* **9**, 142 (2006).
19. C. Jørgensen *et al.*, *Science* **318**, 1247 (2007).
20. D. P. Swain, R. K. Mohn, C. T. Marshall, *Can. J. Fish. Aquat. Sci.* **69**, 997 (2012).
21. Details on methods and data sources are available as supplementary material on Science Online.
22. A. Kuparinen, J. A. Hutchings, *Proc. Biol. Sci.* **279**, 2571 (2012).
23. K. Enberg, C. Jørgensen, E. S. Dunlop, M. Heino, U. Dieckmann, *Evol. Appl.* **2**, 394 (2009).
24. D. M. T. Sharpe, A. P. Hendry, *Evol. Appl.* **2**, 260 (2009).
25. Report of the 2012 Atlantic Bluefin Tuna Stock Assessment Session, Madrid, Spain, 4 to 11 September 2012; www.iccat.int/Documents/Meetings/Docs/2012_BFT_ASSESS.pdf
26. C. J. Walters, R. Hilborn, V. Christensen, *Can. J. Fish. Aquat. Sci.* **65**, 2536 (2008).
27. R. Hilborn, E. Litzinger, *The Open Fish Science Journal* **2**, 32 (2009).
28. J. F. Caddy, D. J. Agnew, *Rev. Fish Biol. Fish.* **14**, 43 (2004).
29. C. A. Holt, A. E. Punt, *Fish. Res.* **100**, 57 (2009).

Acknowledgments: We acknowledge financial support from NFS/National Oceanic and Atmospheric Administration Comparative Analysis of Marine Ecosystem Organization (CAMEO) grant 1041678, Natural Sciences and Engineering Research Council Discovery Grants to J.A.H. and J.K.B., and a Sloan Fellowship to J.K.B. We thank R. Hilborn, T. Branch, and the Jensen Laboratory group for insightful comments. Comments by three anonymous reviewers substantially improved the manuscript. Source data are available at <http://ramlegacy.marinebiodiversity.ca>.

Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6130/347/DC1
Figs. S1 to S7
Tables S1 and S2
References (30–42)

19 September 2012; accepted 4 March 2013
10.1126/science.1230441

Fig. 3. Rebuilding expectations for currently depleted stocks. (A) Ratio of predicted rebuilding times without fishing for depleted stocks to theoretical rebuilding times as predicted by a Graham-Schaefer model (12), as a function of historic fishing intensity and exploitation time. (B) Expected rebuilding times for depleted stocks fished at F_{MSY} , as a function of intrinsic rate of increase (r) and depletion level, and all other variables set to the mean values for currently depleted stocks. In each panel, black crosses denote currently depleted individual stocks, shown at current levels of the plotted variables.

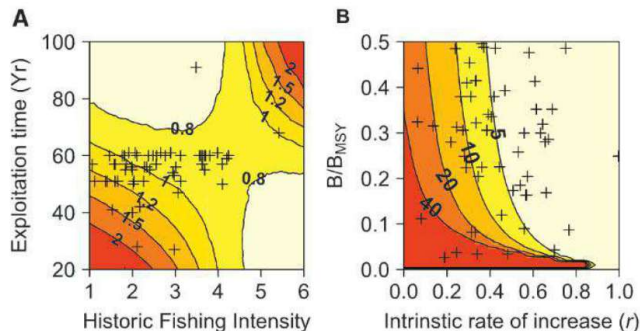
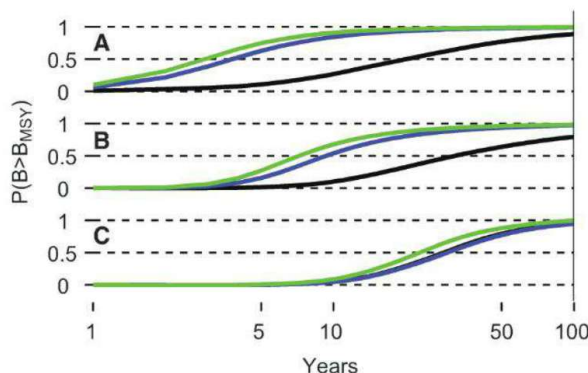


Fig. 4. Predicted (cumulative) recovery probability as a function of time (log number of years). Panels show predictions for (A) Eastern Atlantic bluefin tuna (*T. thynnus*), (B) Irish Sea Atlantic cod (*G. morhua*), and (C) Georges Bank/Gulf of Maine Atlantic halibut (*H. hippoglossus*) at mean fishing mortality rates over the last 3 years in the time series (black lines), fishing at F_{MSY} (blue lines), and no fishing (green lines). Greater spread in the cumulative probability along the x axis corresponds to greater uncertainty in (log) recovery times.



A KRAB/KAP1-miRNA Cascade Regulates Erythropoiesis Through Stage-Specific Control of Mitophagy

Isabelle Barde,¹ Benjamin Rauwel,¹ Ray Marcel Marin-Florez,^{2*} Andrea Corsinotti,^{1†} Elisa Laurenti,^{1‡} Sonia Verp,¹ Sandra Offner,¹ Julien Marquis,^{1§} Adamandia Kapopoulou,¹ Jiri Vanicek,² Didier Trono^{1||}

During hematopoiesis, lineage- and stage-specific transcription factors work in concert with chromatin modifiers to direct the differentiation of all blood cells. We explored the role of KRAB-containing zinc finger proteins (KRAB-ZFPs) and their cofactor KAP1 in this process. In mice, hematopoietic-restricted deletion of *Kap1* resulted in severe hypoproliferative anemia. *Kap1*-deleted erythroblasts failed to induce mitophagy-associated genes and retained mitochondria. This was due to persistent expression of microRNAs (miRNAs) targeting mitophagy transcripts, itself secondary to a lack of repression by stage-specific KRAB-ZFPs. The KRAB/KAP1-miRNA regulatory cascade is evolutionarily conserved, as it also controls mitophagy during human erythropoiesis. Thus, a multilayered transcription regulatory system is present, in which protein- and RNA-based repressors are superimposed in combinatorial fashion to govern the timely triggering of an important differentiation event.

Through the process of erythropoiesis, about 100 billion new red cells are generated every day in the human adult bone marrow. This process is initiated by the differentiation of hematopoietic stem cells (HSCs) into the earliest erythroid progenitor, which was identified *ex vivo* as a slowly growing burst-forming unit. This erythroid progenitor morphs into the rapidly dividing colony-forming unit, the proliferation of which is stimulated by the hypoxia-induced hormone erythropoietin. Further differentiation occurs

through a highly sophisticated program orchestrated by lineage- and stage-specific combinations of protein- and RNA-based transcription regulators (1–3). It culminates in the elimination of intracellular organelles (including mitochondria and the nucleus) to yield the fully mature erythrocyte, containing on the order of 250 million molecules of hemoglobin as almost sole cargo. Much is still to be learned about the molecular mechanisms of these events, not only to understand the cause of red cell disorders, but also to

aid the *in vitro* manufacturing of large supplies of oxygen-carrying cells for transfusion.

The genomes of higher vertebrates encode hundreds of KRAB-containing zinc finger proteins (KRAB-ZFPs) that can bind DNA in a sequence-specific fashion through a C-terminal array of C₂H₂ zinc fingers and can recruit the co-repressor KAP1 via their N-terminal KRAB domain (4–7). KAP1, also known as TRIM28 (tripartite motif protein 28), TIF1 β (transcription intermediary factor 1 β), or KRIP-1 (KRAB-interacting protein 1), acts as a scaffold for a multimolecular complex that silences transcription through the formation of heterochromatin (8–11). The KRAB/KAP1 system probably evolved initially to minimize retroelement-induced genome perturbations (12–14), but recent data indicate that it also regulates multiple aspects of mammalian physiology (15–24). The present study was undertaken to explore its role in hematopoiesis.

¹School of Life Sciences and Frontiers in Genetics Program, Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland. ²School of Basic Sciences, EPFL, 1015 Lausanne, Switzerland.

*Present address: Center for Integrative Genomics, University of Lausanne, 1015 Lausanne, Switzerland.

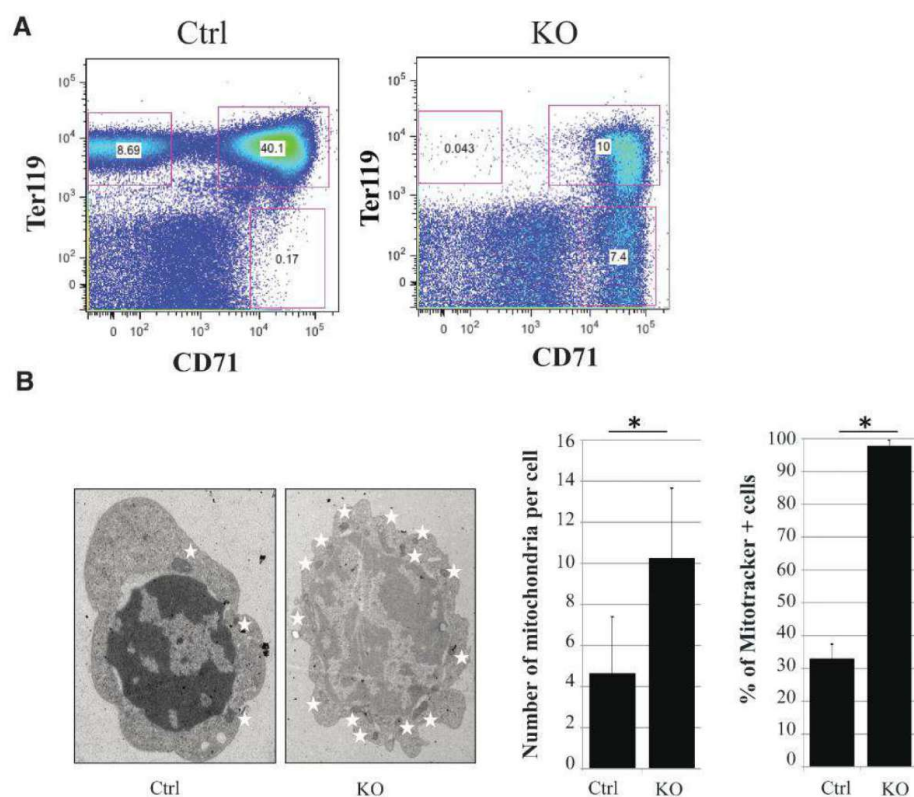
†Present address: Centre for Genomic Regulation, 08003 Barcelona, Spain.

‡Present address: Campbell Family Institute for Cancer Research, Ontario Cancer Institute, Princess Margaret Cancer Centre, University Health Network and Department of Molecular Genetics, University of Toronto, Toronto, Ontario M5S 1A8, Canada.

§Present address: Functional Genomics Core, Nestlé Institute of Health Sciences, EPFL Campus, 1015 Lausanne, Switzerland.

||Corresponding author. E-mail: didier.trono@epfl.ch

Fig. 1. Blocked erythrocyte maturation and accumulation of mitochondria in *Kap1*-deleted erythroblasts. (A) Fluorescence-activated cell sorting (FACS) analysis of CD71 and Ter119 in bone marrow from control (Ctrl) and *Kap1* KO mice 7 weeks after polyinosinic-polycytidylic acid injection. Percentage of each population from the total bone marrow is indicated. (B) Electron microscopy (left, stars indicate mitochondria; center, average number of mitochondria visualized per cell; $n = 10$, $*P < 0.05$) and MitoTracker staining (right, $n = 4$, $*P < 0.05$). Here and in other figures, error bars denote SD. Decreased nuclear density was frequent in *Kap1* KO cells, perhaps reflecting altered chromatin condensation.



The hemato-specific knockout of *Kap1* in the mouse, whereby the hematopoietic system of otherwise wild-type animals is reconstituted

from *Kap1*-deleted hematopoietic stem cells and progenitors (fig. S1), resulted in a series of hematological abnormalities (table S1). Mutant

mice displayed fatal hyporegenerative anemia, characterized by the accumulation of transferrin receptor/CD71⁺ glycophorin A-associated/Ter119⁺

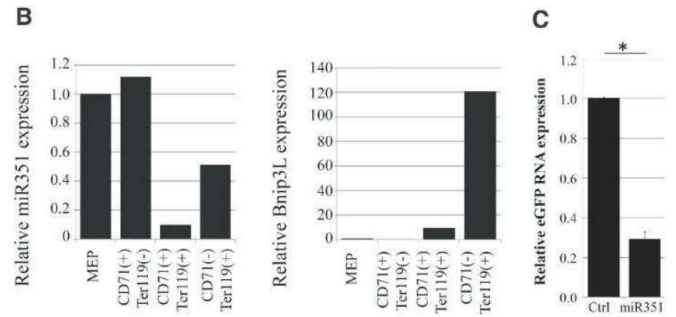
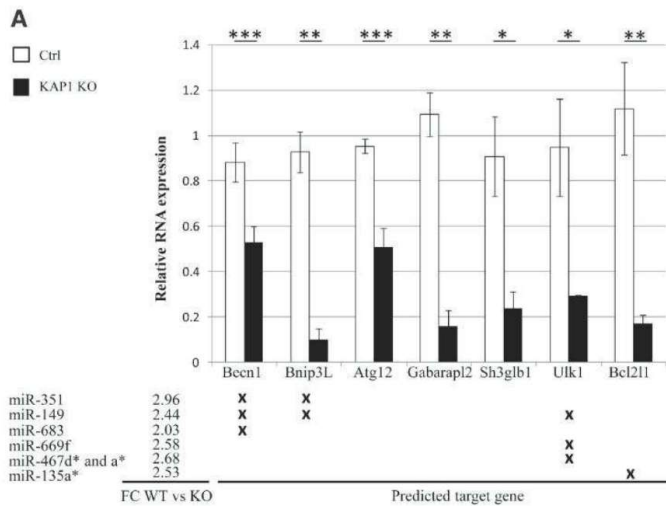


Fig. 2. A KAP1-miRNA cascade controls red cell mitophagy. (A) Top: Mitophagy-related transcripts in erythroblasts from control (Ctrl) and *Kap1* KO mice ($n = 4$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). Bottom: Indicated miRNA expression in the same samples; X denotes predicted miRNA-target pairs. (B) miR-351 and Bnip3L expression in megakaryocyte/erythroid progenitors (MEP: Lin⁻ Sca1⁻ CD117⁺ CD34⁻ CD16.32⁻, in which expression was set at 1) and indicated erythroblast subsets. (C) miR-351 targets the *Bnip3L* 3'UTR.

Ctrl, for which the normalized value was set at 1, was a combination of MEL cells not overexpressing miR-351 and transduced with a GFP-expressing lentiviral vector with the *Bnip3L* 3'UTR, and cells overexpressing miR-351 but transduced with the same vector without this sequence ($n = 3$, $*P < 0.05$).

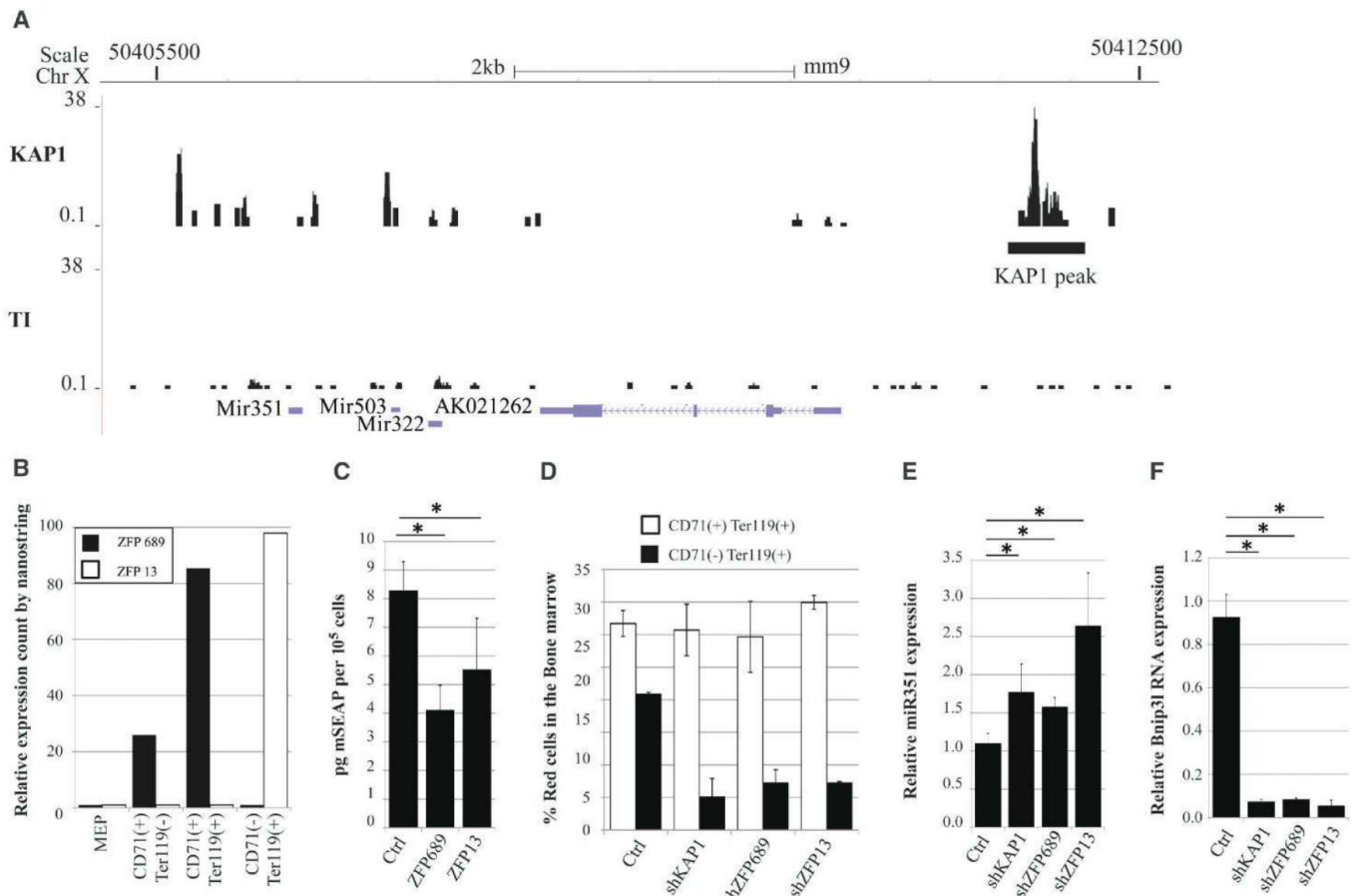


Fig. 3. Erythroblast-specific KRAB-ZFPs control the miR-351-Bnip3L-mitophagy axis. (A) Screen shots from the UCSC Genome Browser, with results of a KAP1 ChIP-seq analysis performed on CD71⁺ Ter119⁺ bone marrow cells. (B) *Zfp689* and *Zfp13* are induced during erythroid differentiation. (C) ZFP689 and ZFP13 repress a lentiviral vector carrying a miR-351-close KAP1-binding site in transduced MEL cells (Ctrl is a combination of ZFP-overexpressing cells transduced with a vector without the KAP1-binding site and LacZ-overexpressing

cells transduced with a vector carrying the KAP1-binding site; $n = 3$, $*P < 0.05$). (D) CD45.2⁺ LSK cells were transduced with GFP-expressing, empty or scramble (Ctrl), *Kap1*-, *Zfp689*-, or *Zfp13*-directed shRNA lentiviral vectors, engrafted into irradiated CD45.1⁺ mice; erythroid differentiation was evaluated by FACS 8 weeks later. (E and F) The CD71⁺ Ter119⁺ CD45.2⁺ eGFP⁺ population was then sorted and analyzed by reverse transcription quantitative polymerase chain reaction (RT-qPCR) for miR-351 (E) and Bnip3L (F) expression ($n = 6$, $*P < 0.05$).

early erythroblasts and an almost complete absence of mature CD71⁺ Ter119⁺ cells in the bone marrow (Fig. 1A). Electron microscopy and MitoTracker staining revealed that *Kap1* KO erythroblasts contain more mitochondria than their wild-type counterparts (Fig. 1B), correlating with decreased expression of mitophagy transcripts such as *Nix/BNIP3L*, *Ulk1*, *Gabrarip2*, *Sh3glb1*, *Atg12*, *Becn1*, and *Bcl2l1* (Fig. 2A). Because the KRAB/KAP1 pathway is mostly known to induce transcriptional repression (10, 11), it seemed likely that this effect was indirect. An examination of the microRNA (miRNA) expression profile of control and *Kap1* KO CD71⁺ Ter119⁺ cells revealed that among 455 miRNAs tested, 5 were down-regulated and 11 up-regulated by more than a factor of 2 in KO cells (data are presented in the Gene Expression Omnibus data set GSE44061).

A recently described *in silico* approach (25, 26) suggested that six of these up-regulated miRNAs had mitophagy-associated deregulated transcripts as their targets, notably miR-351, predicted to act on *BNIP3L* (Fig. 2A). Consistent with this hypothesis, levels of miR-351 abruptly dropped in CD71⁺ Ter119⁺ cells relative to their CD71⁺ Ter119⁺ precursors, mirroring *BNIP3L* induction (Fig. 2B). Furthermore, transduction of mouse erythroleukemia (MEL) cells with a green fluorescent protein (GFP)-expressing lentiviral vector harboring 3' of GFP, the *BNIP3L* 3' untranslated region (3'UTR) sequence predicted to be targeted by miR-351 resulted in miR-351-dependent down-regulation of the reporter (Fig. 2C). Finally, similar to their KAP1-depleted counterparts, miR-351-overexpressing MEL cells were blocked in differentiation and accumulated mitochondria, and this phenotype was reversed by expression of a *BNIP3L* transcript devoid of this 3'UTR sequence (fig. S2).

Both miR-503 and miR-322, which are located next to miR-351 on chromosome X, were also up-regulated (by factors of 2.46 and 2.17, respectively) in *Kap1* KO erythroblasts. Consistent with a role for KRAB/KAP1 in regulating this miRNA gene cluster, chromatin immunoprecipitation coupled to DNA sequencing (ChIP-seq) detected a strong KAP1 peak less than 4 kb away (Fig. 3A). Because KAP1 is not a DNA binding protein, we postulated that it might be tethered to this and other relevant loci by stage-specific KRAB-ZFPs. Nine KRAB-ZFP genes were identified, which had human orthologs and were expressed exclusively in CD71⁺ Ter119⁺ and/or CD71⁺ Ter119⁺ erythroblasts but not in other hematopoietic cells. Six of these genes could be efficiently knocked down in MEL cells by lentivector-mediated RNA interference, and two of them, *Zfp689* and *Zfp13*, emerged as potential *BNIP3L* regulators (fig. S3). Interestingly, *Zfp689* is expressed in CD71⁺ Ter119⁺ erythroblasts, whereas *Zfp13* is expressed only in their CD71⁺ Ter119⁺ counterparts (Fig. 3B). Both could repress reporter expression in MEL cells transduced with a lentiviral vector harboring the miR-351-close KAP1-binding site upstream of a human

phosphoglycerate kinase promoter mSEAP (murine secreted alkaline phosphatase) cassette (Fig. 3C). We then validated these two candidates *in vivo* by transplanting CD45.2 hematopoietic stem cells [lineage[−], *Scal*⁺, and cKit⁺ (LSK)] transduced with lentiviral vectors producing GFP and short hairpin RNAs (shRNAs) against *Zfp689*, *Zfp13*, or *Kap1* (control) into irradiated CD45.1 mice, allowing the dual discrimination of donor versus recipient and transduced versus untransduced cells. Analyses of the red cell compartment in bone marrow harvested 8 weeks after the graft revealed that knockdown of either *Zfp689* or *Zfp13* led to a decrease in CD71⁺ Ter119⁺ cells as pronounced as that observed with the *Kap1* knockdown (Fig. 3D). Furthermore, RNA analyses of sorted transduced CD71⁺ Ter119⁺ cells demonstrated that ZFP689-, ZFP13-, and KAP1-depleted cells all exhibited an up-regulation of miR-351 (Fig. 3E) and a marked down-regulation of *BNIP3L* (Fig. 3F).

In a last series of experiments, we asked whether this erythropoiesis-regulating system has an equivalent in humans. We first found that *Kap1* knockdown impaired the differentiation of human erythroleukemia (HEL) cells and increased their mitochondrial content (Fig. 4, A to C), blocking several mitophagy effectors including *Nix/BNIP3L* (Fig. 4D). We further verified that KAP1-depleted HEL cells had increased levels of hsa-miR-125a-5p (Fig. 4D), which has the same miRNA seed as murine miR-351, and that overexpression of this miRNA triggered a down-regulation of *Nix* and a rise in the mitochondrial content of these cells (Fig. 4E). Finally, when we knocked down

Kap1 in human cord blood CD34⁺ cells, it resulted in a decrease of their ability to undergo cytokine-induced *ex vivo* erythroid differentiation, which correlated with reduced *Nix* expression and elevated mitochondrial content (Fig. 4F)—a phenotype that could be reproduced by hsa-miR-125a overexpression (Fig. 4G).

These results indicate the presence of a multi-layered transcription regulatory system in which protein- and RNA-based repressors are superimposed in combinatorial fashion to govern the timely triggering of a necessary step of erythropoiesis. miR-351 and several other miRNAs with predicted targets in the mitophagy pathway were up-regulated in *Kap1*-deleted murine erythroblasts (Fig. 2). This apparent redundancy—or, rather, addition of parallel effects aimed at the same physiological process—is commonly observed with RNA interference (27). Our discovery that it can be further modulated by KRAB-ZFP-mediated repression, and that the latter can itself be multifactorial, adds a remarkable level of modularity to this type of regulation. In human erythroblasts, although KAP1 represses the *Nix*-targeting hsa-miR-125a-5p, down-regulation of several other miRNAs (including hsa-miR-24, -221, -222, and -223) was previously found to be important for erythroid differentiation, which conversely requires the up-regulation of the hsa-miR-144/451 cluster (2, 3). Whether stage-specific KRAB-ZFPs are involved in controlling some of these other miRNAs remains to be determined. Even though KAP1 likely influences erythropoiesis by more than just allowing mitophagy, it is interesting to note that *Znf205* and *Znf689*, the respective

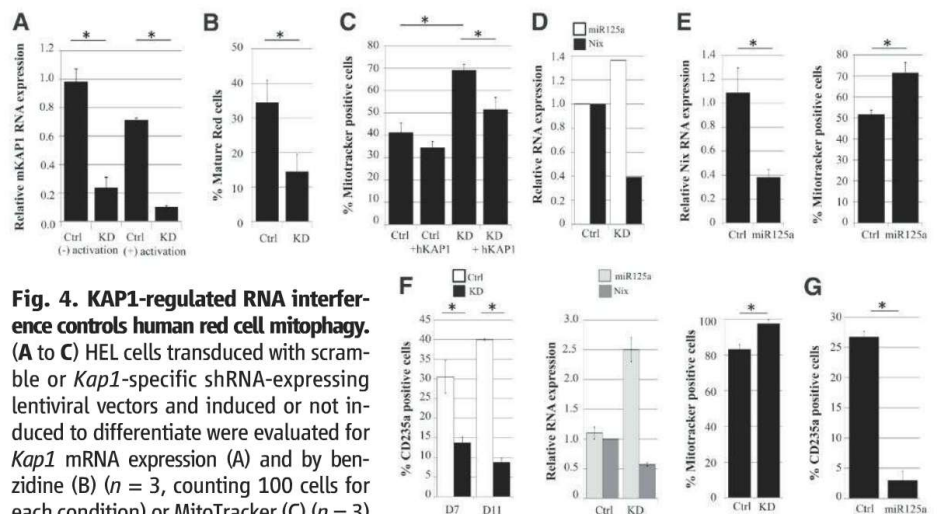


Fig. 4. KAP1-regulated RNA interference controls human red cell mitophagy. (A to C) HEL cells transduced with scramble or *Kap1*-specific shRNA-expressing lentiviral vectors and induced or not induced to differentiate were evaluated for *Kap1* mRNA expression (A) and by benzidine (B) ($n = 3$, counting 100 cells for each condition) or MitoTracker (C) ($n = 3$) staining ($*P < 0.05$). (D) hsa-miR-125a-5p (miR125a) and *Nix* expression measured respectively by NanoString nCounter direct RNA quantification and RNA sequencing in HEL cells transduced with empty or *Kap1* knockdown vectors. (E) *Nix* expression in Ctrl (setting normalized value at 1) or hsa-miR-125a-5p-overexpressing HEL cells, measuring their mitochondrial content by MitoTracker staining ($n = 4$, $*P < 0.05$). (F) Decreased erythroid differentiation of *Kap1* knockdown human cord blood CD34⁺ cells, assessed by CD235a surface expression at 7 and 11 days (D7, D11). At D7, sorted CD235a⁺ eGFP⁺ cells were analyzed by RT-qPCR for *Nix* and hsa-miR-125a expression, and for mitochondrial content by MitoTracker staining ($n = 3$, $*P < 0.05$). (G) Percentage of CD235a-expressing cells 7 days after inducing the differentiation of CD34⁺ cells transduced with empty or unrelated miRNA-expressing (Ctrl) or hsa-miR-125a-5p-overexpressing lentiviral vectors ($n = 3$, $*P < 0.05$).

human orthologs of murine *Zfp13* and *Zfp689*, are expressed in HEL cells and induced upon erythroid differentiation of CD34⁺ cells (fig. S4). Therefore, polymorphism or mutations in any genetic component of the pathway unveiled here—whether *Znf205*, *Znf689*, the genomic binding sites of their products, hsa-miR-125a-5p and other KAP1-regulated miRNA genes, or the sequences targeted by these RNA regulators—could underlie red cell-related pathologies such as anemia, polycythemia, or erythroleukemia.

References and Notes

1. J. Xu et al., *Dev. Cell* **23**, 796 (2012).
2. S. M. Hattangadi, P. Wong, L. Zhang, J. Flygare, H. F. Lodish, *Blood* **118**, 6258 (2011).
3. C. H. Lawrie, *Br. J. Haematol.* **150**, 144 (2010).
4. E. J. Bellefroid, D. A. Poncelet, P. J. Lecocq, O. Revelant, J. A. Martial, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 3608 (1991).
5. J. M. Vaquerizas, S. K. Kummerfeld, S. A. Teichmann, N. M. Luscombe, *Nat. Rev. Genet.* **10**, 252 (2009).
6. J. C. Venter et al., *Science* **291**, 1304 (2001).
7. A. T. Chinwalla et al., *Nature* **420**, 520 (2002).
8. A. L. Nielsen et al., *EMBO J.* **18**, 6385 (1999).
9. S. Quenneville et al., *Cell Rep.* **2**, 766 (2012).
10. D. C. Schultz, K. Ayyanathan, D. Negorev, G. G. Maul, F. J. Rauscher 3rd, *Genes Dev.* **16**, 919 (2002).
11. D. C. Schultz, J. R. Friedman, F. J. Rauscher 3rd, *Genes Dev.* **15**, 428 (2001).
12. H. M. Rowe et al., *Nature* **463**, 237 (2010).
13. H. M. Rowe et al., *Genome Res.* **23**, 452 (2012).
14. D. Wolf, S. P. Goff, *Cell* **131**, 46 (2007).
15. K. Bojkowska et al., *Hepatology* **56**, 1279 (2012).
16. S. Chikuma, N. Saita, I. M. Okazaki, S. Shibayama, T. Honjo, *Nat. Immunol.* **13**, 596 (2012).
17. J. Jakobsson et al., *Neuron* **60**, 818 (2008).
18. X. Li et al., *Dev. Cell* **15**, 547 (2008).
19. D. J. Mackay et al., *Nat. Genet.* **40**, 949 (2008).
20. F. R. Santoni de Sio et al., *FASEB J.* **10.1096/fj.12-206177** (2012).
21. F. R. Santoni de Sio et al., *Blood* **119**, 4675 (2012).
22. J. H. Shin et al., *Cell* **144**, 689 (2011).
23. L. Zheng et al., *Mol. Cell* **6**, 757 (2000).
24. Y. Ziv et al., *Nat. Cell Biol.* **8**, 870 (2006).
25. R. M. Marín, J. Vaníček, *Nucleic Acids Res.* **39**, 19 (2011).
26. R. M. Marín, J. Vaníček, *PLoS ONE* **7**, e32208 (2012).
27. M. S. Ebert, P. A. Sharp, *Cell* **149**, 515 (2012).

Acknowledgments: We thank the staff of our core facilities for technical expertise, and P. Turelli, B. Yazdanpanah, and F. Marzetta (EPFL), P. Malik (Cincinnati Children's Hospital), and G. Ferrari (San Raffaele Telethon Institute for Gene Therapy, Milano) for the kind gift of reagents. Supported by grants from the Swiss National Science Foundation and the European Research Council (D.T.). The cDNA and miRNA microarray and ChIP-seq data are deposited in the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo) under accession number GSE44061. D.T. and I.B. are inventors on a patent application submitted by EPFL for the control of mitophagy.

Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1232398/DC1

Materials and Methods

Figs. S1 to S4

Table S1

References (28–38)

5 November 2012; accepted 26 February 2013

Published online 14 March 2013;

10.1126/science.1232398

The Helicase-Like Domains of Type III Restriction Enzymes Trigger Long-Range Diffusion Along DNA

Friedrich W. Schwarz,^{1*} Júlia Tóth,^{2*} Kara van Aelst,² Guanshen Cui,² Sylvia Clausing,¹ Mark D. Szczelkun,^{2†} Ralf Seidel^{1,3‡}

Helicases are ubiquitous adenosine triphosphatases (ATPases) with widespread roles in genome metabolism. Here, we report a previously undescribed functionality for ATPases with helicase-like domains; namely, that ATP hydrolysis can trigger ATP-independent long-range protein diffusion on DNA in one dimension (1D). Specifically, using single-molecule fluorescence microscopy we show that the Type III restriction enzyme EcoP15I uses its ATPase to switch into a distinct structural state that diffuses on DNA over long distances and long times. The switching occurs only upon binding to the target site and requires hydrolysis of ~30 ATPs. We define the mechanism for these enzymes and show how ATPase activity is involved in DNA target site verification and 1D signaling, roles that are common in DNA metabolism: for example, in nucleotide excision and mismatch repair.

Helicases were defined classically as adenosine triphosphatases (ATPases) that use directional translocation to unwind duplex nucleic acids. More recently, however, many “pseudo-helicases” have been described that possess amino acid motifs characteristic of helicases yet fulfill their cellular functions without obligate DNA unwinding (1) and instead are often translocases that move directionally on single- (2) or double-stranded (3, 4) nucleic acids. Pseudo-helicases include the ATP-dependent bacterial Type I and III restriction enzymes (REs), which use ATP hydrolysis to communicate between two distant restriction sites on the same DNA (5, 6).

Only if both sites are unmethylated is the DNA considered “foreign” and destroyed by the endonuclease. Type III REs form a heterooligomeric complex of two subunits: Mod (for target site methylation) and Res (for ATPase and endonuclease activities). Because they only hemimethylate their asymmetric recognition sequences, these enzymes must signal the relative site orientation during communication (7): Cleavage occurs only if the sites are in an inverted-repeat orientation, arranged either “head-to-head” (HtH) (7) or “tail-to-tail” (TtT) (8). Type III REs hydrolyze only a few tens of ATPs per double-stranded DNA (dsDNA) break (9, 10), suggesting either passive three-dimensional (3D) looping between sites with limited translocation (11) or diffusion in 1D following an ATP-dependent switch (fig. S1A) (8, 10).

To distinguish between the different communication models, we developed a real-time single-molecule assay using magnetic tweezers combined with total internal reflection fluorescence (TIRF)

microscopy (12) that can localize fluorescently labeled enzymes on stretched 26-kilobase pair (kbp) DNA molecules containing two centrally located HtH EcoP15I sites with 6-kbp intersite distance (Fig. 1A). EcoP15I was labeled with quantum dots on the C terminus of the Res subunit (QR-EcoP15I), without compromising the biochemical activity (fig. S2). Whereas DNA binding by lone quantum dots was not detected, addition of QR-EcoP15I and ATP resulted in enzyme attachment at one (Fig. 1B and movie S1) or two (fig. S3) specific recognition sites. Enzymes always arrived instantaneously at the sites, with no evidence for long-lived, nonspecific binding en route ($N = 61$). After a delay of, on average, 17 ± 2 s (fig. S4), bound enzymes started to move rapidly in both directions along the DNA (Fig. 1B), consistent with random walk simulations using measured parameters (13) (fig. S5). All bound enzyme complexes ($N = 91$) could be assigned to one of the two target sites (fig. S6A), and 90% exhibited fast bidirectional movement. The mean-square displacement determined from single-particle tracking time trajectories (14) was found to increase linearly with time, confirming a 1D diffusion with a diffusion constant $D = 0.92 \pm 0.06 \mu\text{m}^2 \text{s}^{-1}$ (Fig. 1C). This is one of the highest values reported, suggesting that EcoP15I is optimized for rapid, long-distance DNA scanning (supplementary text). Rebinding to the original site or a second distant specific site was often observed, after which additional sliding events could restart with a similar initiation rate (fig. S4) until final dissociation occurred. Rebinding occurred preferentially at sites with the same orientation as the start site (Fig. 1D and fig. S4). The average lifetime of a diffusion event was 9 ± 1 s, whereas the average total DNA interaction time involving multiple sliding and rebinding events was 180 ± 10 s (fig. S4). Thus, an enzyme can explore thousands of base pairs up- and downstream of the original site before dissociation. On a substrate with a sufficient density of EcoP15I

¹DNA motors group, Biotechnology Center, Technische Universität Dresden, 01062 Dresden, Germany. ²DNA-Protein Interactions Unit, School of Biochemistry, Medical Sciences Building, University of Bristol, Bristol BS8 1TD, UK. ³Institute of Molecular Cell Biology, University of Münster, 48149 Münster, Germany.

*These authors contributed equally to this work.

†Corresponding author. E-mail: mark.szczelkun@bristol.ac.uk (M.D.S.); ralf.seidel@uni-muenster.de (R.S.)

sites, DNA cleavage was observed after collisions between a mobile enzyme and a site-bound enzyme (fig. S6B). Given the diffusion constant and the viscous drag of the quantum dot, QR-EcoP15I cannot follow a helical path along the DNA (15) (supplementary text). We interpret this to mean that EcoP15I can move on DNA independent of the helical structure, as might be explained by a proposed toroidal clamp configuration (16). Supporting such a structure is the observation that protein roadblocks at DNA ends increase endonuclease efficiency as they prevent dissociation by sliding (10).

The period of stable target-site binding before initiation of diffusion suggested the existence of a conformational switch, because enzymes binding the DNA from an ATP solution did not directly enter the diffusive state. To determine whether ATP hydrolysis drives the switching, we repeated our measurements in the absence (apo) or presence of different nucleotide analogs: nonhydrolyzable adenylyl-imidodiphosphate (AMP-PNP); the transition-state mimic adenosine 5'-diphosphate (ADP)- Va^{2+} ; and ADP. In contrast to ATP, in all cases we observed numerous nonspecific association events (Fig. 2A). For apo, AMP-PNP, and ADP- Va^{2+} conditions, QR-EcoP15I was almost immobile. For ADP, nonspecific binding was combined with occasional excursions into long-lived diffusion states, albeit with a diffusion constant 1/30 of the value observed in ATP (fig. S7). To examine the effect of ATP on nonspecific complexes, we prebound QR-EcoP15I in the apo conditions for a fixed period. After flushing in ATP, most prebound enzymes dissociated from the DNA. The remaining enzymes slowly diffused to a target site and, once specifically bound, started to undergo cycles of fast diffusive motion and target-site rebinding as before (Fig. 2B). Thus, ATP hydrolysis not only initiates fast diffusion from specific sites but also reduces nonspecific binding, thereby increasing specificity.

We measured DNA binding and ATP consumption by EcoP15I using stopped-flow fluorimetry (17, 18). Prebinding EcoP15I to a short specific oligonucleotide, and then rapidly mixing with an enzyme trap, allowed measurement of enzyme-DNA dissociation. The kinetics were most rapid with ATP (6.6 ± 0.1 s lifetime on DNA, fig. S8), of intermediate velocity with ADP and ADP + P_i (inorganic phosphate), and appreciably slower with AMP-PNP, P_i , or apo conditions (Fig. 2C), in qualitative agreement with the single-molecule measurements. ATP hydrolysis was measured under the same reaction configuration (Fig. 2D). On specific DNA only, hydrolysis of ~ 30 ATP molecules was coupled to each release event with ~ 10 ATPs consumed within an initial 1-s burst followed by ~ 20 ATPs at a lower rate, similar to the accompanying dissociation kinetics (Fig. 2D and fig. S8). Tryptophan fluorescence changes during dissociation point to a single-step nucleoprotein remodeling event coupled to the initial 10 ATPs (fig. S9), whereas

short-range translocation events [i.e., “DNA scrunching” (19)] were not observed (fig. S10). ADP did not produce the same changes in tryptophan fluorescence, consistent with a different sliding conformation.

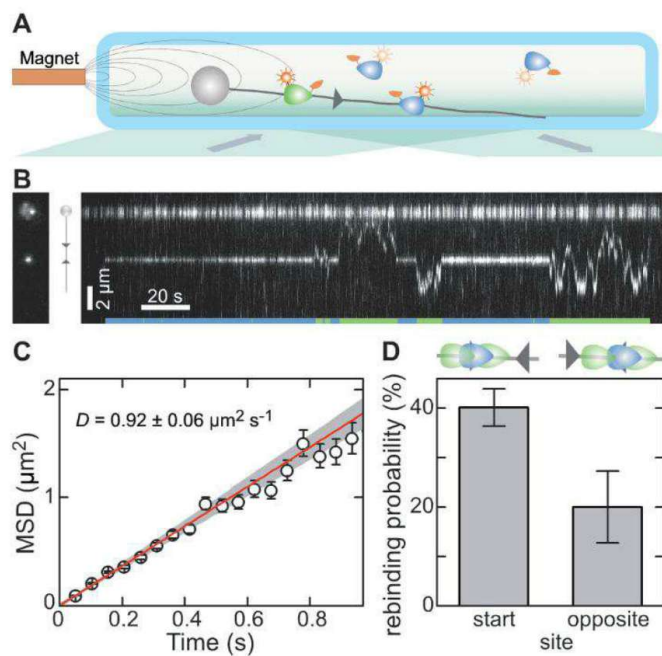
To address whether ATP is required during sliding, we measured ATP hydrolysis using DNA of different lengths, with and without streptavidin roadblocks at the ends (Fig. 3A and fig. S11). End-capping prevents EcoP15I sliding off the DNA and increases the DNA-bound lifetime (~ 200 s) compared to uncapped DNA (< 1 s) (20). There are two distinct stages in the ATPase profiles: a burst phase, due to site-specific ATP hydrolysis and initiation of sliding; and a steady-state phase, due to enzymes binding from solution and initiating sliding (consuming ~ 30 ATPs) and any other ATPase activity during sliding. If appreciable amounts of ATP were consumed during sliding or upon site rebinding, we might expect a lower steady-state rate on uncapped DNA than on capped DNA because of the longer sliding lifetime on the latter substrate. Instead we observed little difference in the steady-state rates and little dependence on DNA length, suggesting that negligible ATP is hydrolyzed during diffusion or after site rebinding.

We also measured the nucleotide dependence of site-specific binding by EcoP15I (Fig. 3B). Whereas saturated binding was observed with apo, AMP-PNP, and ADP conditions, binding in ATP was characterized by a transient maximum followed by a lower steady state. A two-state model (free and bound) cannot describe this pro-

file. However, satisfactory fits were obtained by introducing a third state (in agreement with the tryptophan fluorescence measurements) after ATP-dependent DNA dissociation, where an activated EcoP15I must slowly re-isomerize before it can rebind DNA (Fig. 3C and fig. S12). The target-site-binding lifetime of 5.9 ± 0.7 s matches that observed in Fig. 2D and is similar to the value from the single-molecule experiments. The activated state has a shorter lifetime (~ 25 s) than the total interaction time measured in the tweezers, but nonetheless we interpret this as representing the sliding conformation—the shorter observed lifetime may reflect the absence of bound DNA.

In this work, we provide direct evidence for the 1D diffusion hypothesis for intersite communication and cleavage by Type III REs (fig. S1B). One-dimensional diffusion on DNA is a general model for target-site localization by proteins (21), but sliding events in this situation are short-lived and can be interspersed by microscopic dissociation-reassociation events (“hopping”). Hopping can be ruled out for Type III REs as this would lose the original asymmetric binding orientation and would negate the site-orientation selectivity of these enzymes. The EcoP15I mechanism highlights that long-lived sliding is only achieved by a conformational switch catalyzed by site-dependent ATP hydrolysis. The necessity for an ATP hydrolysis step to establish long-lived sliding has precedent in the loading of processivity clamps at replication forks by the AAA+ family clamp loader complexes (22). There are also close

Fig. 1. Direct observation of long-range diffusion on DNA by the Type III restriction enzyme EcoP15I in the presence of ATP. (A) Detection scheme based on magnetic tweezers that allows simultaneous DNA stretching and TIRF microscopy of quantum dot-labeled enzymes. (B) Kymographs of the interaction of 0.7 nM QR-EcoP15I in the presence of ATP with a DNA substrate that contains two target sites in an inverse HTH orientation, spaced 6 kbp apart (see sketch on the left). The snapshot fluorescence image on the far left shows the magnetic particle and the bound enzyme after 60 s. The colored bar on the bottom of the kymograph highlights periods of enzyme diffusion (green) and target site binding (blue). (C) Cumulative mean-square displacement over time of different diffusion events of EcoP15I on DNA ($N = 32$). A weighted linear fit to the data provides a diffusion constant of $0.92 \pm 0.06 \mu m^2 s^{-1}$ (SEM). (D) Probability of EcoP15I to rebind during the diffusion to its original start site or to the distant target site normalized by the number of attempts ($N = 201$). Error bars in (C) and (D) represent the SEM.



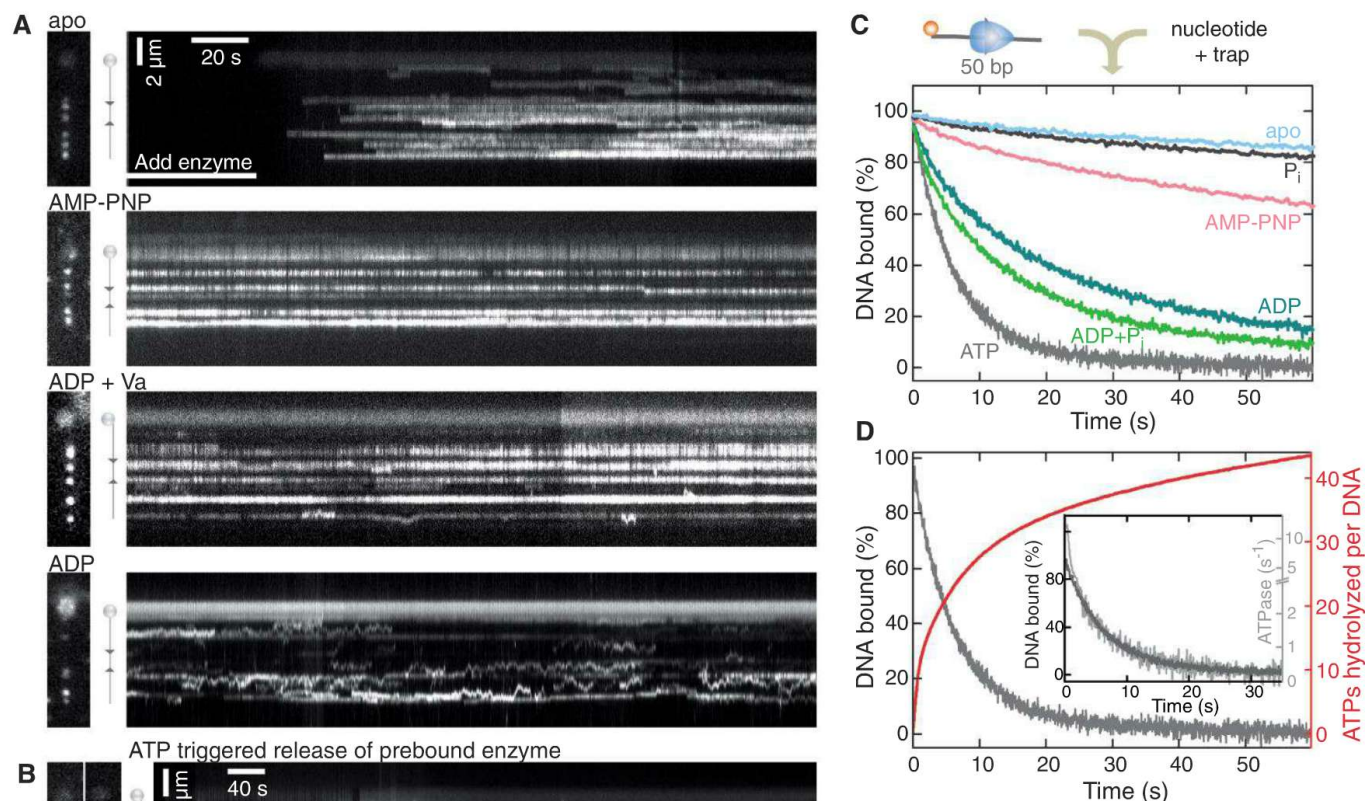
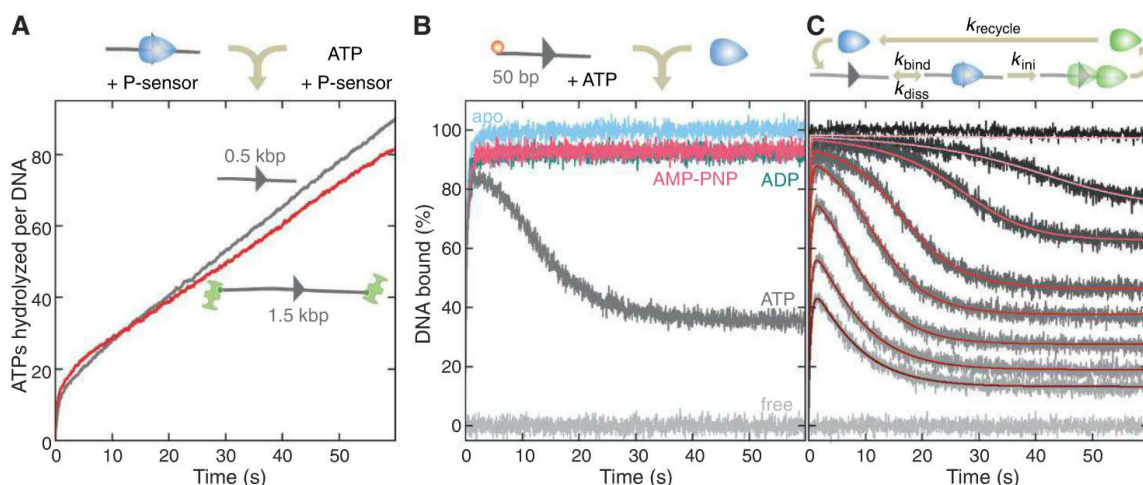


Fig. 2. ATP hydrolysis–triggered release of the enzyme

from its target site. (A) Interaction of 0.13 nM QR-EcoP151 with DNA in the presence of different nucleotides. Apo indicates the absence of any added nucleotide. Images and kymographs have identical scales. Snapshots were taken after 120 s. **(B)** ATP-triggered release of prebound enzyme from DNA. Period 1: Addition of 0.7 nM enzyme in the absence of nucleotide. Period 2: Flush with buffer only. Period 3: Flush with buffer containing ATP. Snapshots shown were taken at 200 s (before ATP addition) and 400 s (after ATP addition). The immobile quantum dot following ATP addition is stuck to the capillary surface in proximity to the DNA. **(C)** Stopped-flow measurements (see sketch) showing the release of prebound enzyme from its target site with different nucleotides. **(D)** Stopped-flow measurements of DNA-dissociation by EcoP151 and the amount of ATP hydrolyzed upon adding ATP and heparin. The inset shows the DNA dissociation kinetics and the instantaneous ATPase rate obtained from the derivative of the ATP hydrolysis kinetics (both data from main figure; see fig. S8).

Fig. 3. EcoP151 adopts a new conformation upon ATP hydrolysis–driven target site release. (A)

ATP hydrolysis during target site binding of EcoP151 in the absence of a trap using a short 0.5-kbp DNA with free ends and a longer 1.5-kbp DNA with streptavidin roadblocks at its ends. Stopped-flow measurements were initiated by mixing enzyme prebound to DNA with ATP (see sketch) with both solutions containing a phosphate sensor. **(B)** Binding kinetics of EcoP151 to its target site in the presence or absence of different nucleotides. Stopped-flow anisotropy measurements were initiated by mixing enzyme and DNA supplemented with nucleotide (see sketch). **(C)** Binding kinetics of EcoP151 to its target site in the presence of ATP as measured in (B) for different enzyme concentrations (gray curves; increasing grayscale values correspond to 0, 20,



26, 40, 53, 66, 99, 132, and 265 nM EcoP151). The experimental data were fitted according to the scheme sketched above based on four different rate constants (light to dark red curves). Curves for concentrations of 20 to 132 nM were obtained from fits to the experimental data, whereas mean rate constants were used to simulate the curve for 265 nM (fig. S12).

functional parallels to mismatch repair: After mismatch targeting through helix-correlated sliding and 3D steps, the ABC transporter family MutS enzymes use a two-step conformational change to adopt a sliding clamp configuration that displays accelerated, helix-unrelated 1D diffusion (23–25). This transition requires a sole ADP-to-ATP exchange, contrasting with EcoP15I that hydrolyzes many ATPs upon initiation (fig. S1). Together this shows that ATP-triggered sliding is a general function that has evolved in different ATPase kingdoms.

References and Notes

1. M. R. Singleton, M. S. Dillingham, D. B. Wigley, *Annu. Rev. Biochem.* **76**, 23 (2007).
2. J. Park *et al.*, *Cell* **142**, 544 (2010).
3. L. K. Stanley *et al.*, *EMBO J.* **25**, 2230 (2006).
4. Y. Zhang *et al.*, *Mol. Cell* **24**, 559 (2006).
5. D. T. Dryden, N. E. Murray, D. N. Rao, *Nucleic Acids Res.* **29**, 3728 (2001).
6. N. K. Raghavendra, S. Bheemanaik, D. N. Rao, *Front. Biosci.* **17**, 1094 (2012).
7. A. Meisel, T. A. Bickle, D. H. Krüger, C. Schroeder, *Nature* **355**, 467 (1992).
8. K. van Aelst *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 9123 (2010).
9. A. Meisel, P. Mackeldanz, T. A. Bickle, D. H. Krüger, C. Schroeder, *EMBO J.* **14**, 2958 (1995).
10. S. P. Ramanathan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 1748 (2009).
11. N. Crompton *et al.*, *EMBO J.* **26**, 3815 (2007).
12. H. Brutzer, F. W. Schwarz, R. Seidel, *Nano Lett.* **12**, 473 (2012).
13. F. W. Schwarz, K. van Aelst, J. Tóth, R. Seidel, M. D. Szczelkun, *Nucleic Acids Res.* **39**, 8042 (2011).
14. F. Ruhnnow, D. Zwicker, S. Diez, *Biophys. J.* **100**, 2820 (2011).
15. P. C. Blainey *et al.*, *Nat. Struct. Mol. Biol.* **16**, 1224 (2009).
16. Y. K. Gupta *et al.*, *J. Mol. Biol.* **420**, 261 (2012).
17. M. R. Webb, *Mol. Biosyst.* **3**, 249 (2007).
18. S. E. McClelland, D. T. F. Dryden, M. D. Szczelkun, *J. Mol. Biol.* **348**, 895 (2005).
19. A. Revyakin, C. Liu, R. H. Ebricht, T. R. Strick, *Science* **314**, 1139 (2006).
20. J. Tóth, K. van Aelst, H. Salmons, M. D. Szczelkun, *Nucleic Acids Res.* **40**, 6752 (2012).
21. S. E. Halford, *Biochem. Soc. Trans.* **37**, 343 (2009).
22. B. A. Kelch, D. L. Makino, M. O'Donnell, J. Kuriyan, *Science* **334**, 1675 (2011).
23. W.-K. Cho *et al.*, *Structure* **20**, 1264 (2012).
24. J. Gorman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **109**, E3074 (2012).
25. R. Qiu *et al.*, *EMBO J.* **31**, 2528 (2012).

Acknowledgments: This work was supported by The Wellcome Trust (084086), the Deutsche Forschungsgemeinschaft (DFG, SE 1646/1-1, SE 1646/7-1), a starting grant of the European Research Council (261224), and the Dresden International Graduate School for Biomedicine and Bioengineering, funded by the DFG (to F.W.S.). We thank N. Savery and S. Halford for comments on the manuscript. The authors declare no conflict of interest.

Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6130/353/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S12
References (26–49)

Movie S1

4 October 2012; accepted 22 February 2013
10.1126/science.1231122

Structural Basis for Kinesin-1: Cargo Recognition

Stefano Pernigo,* Anneri Lamprecht,* Roberto A. Steiner,† Mark P. Dodding†

Kinesin-mediated cargo transport is required for many cellular functions and plays a key role in pathological processes. Structural information on how kinesins recognize their cargoes is required for a molecular understanding of this fundamental and ubiquitous process. Here, we present the crystal structure of the tetratricopeptide repeat domain of kinesin light chain 2 in complex with a cargo peptide harboring a "tryptophan-acidic" motif derived from SKIP (SifA-kinesin interacting protein), a critical host determinant in *Salmonella* pathogenesis and a regulator of lysosomal positioning. Structural data together with biophysical, biochemical, and cellular assays allow us to propose a framework for intracellular transport based on the binding by kinesin-1 of W-acidic cargo motifs through a combination of electrostatic interactions and sequence-specific elements, providing direct molecular evidence of the mechanisms for kinesin-1: cargo recognition.

The plus-end-directed motor kinesin-1 plays a critical role in the intracellular transport of diverse protein, ribonuclear protein complexes, and membrane compartments on microtubules (1). Its functions are also usurped by bacteria and viruses to aid in their replication (2, 3). Kinesin-1 can perform this diverse range of functions by virtue of its ability to interact with many different cargo proteins (4). Diversity of cargo recognition is accomplished largely through the kinesin light chains (KLCs), which harbor a tetratricopeptide repeat (TPR) domain, a versatile protein interaction platform (5, 6). The KLC^{TPR} domain can recognize short peptide stretches within relatively disordered regions of its targets. These peptides are characterized by a tryptophan resi-

due flanked by acidic residues (such as EWD) and are found in a growing list of KLC-binding proteins (7–16). [Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. In the mutants, other amino acids were substituted at certain locations; for example, R251D indicates that arginine at position 251 was replaced by aspartic acid.] Although W-acidic motifs often occur in pairs, single motifs are also functional and can support microtubule-based transport even when explanted from their host protein (7, 12, 17).

We set out to solve the structure of a KLC^{TPR} domain bound to a cargo W-acidic motif. We focused our attention on the SifA-kinesin interacting protein (SKIP) cargo for its importance in *Salmonella* pathogenesis. SKIP contains a pair of W-acidic motifs centered at amino acid positions 207 and 208 (WD) and 236 and 237 (WE)

that fall within the N-terminal kinesin-1 binding region (residues 1 to 310) (Fig. 1A) (3, 7, 13). To assess the relative importance of the SKIP W-acidic motifs for KLC binding, we cotransfected HeLa cells with wild-type and WD/WE mutant constructs expressing green fluorescent protein (GFP)-SKIP(1-310) and hemagglutinin (HA)-KLC2 (Fig. 1B).

Disruption of the WD motif reduced GFP-SKIP interaction with HA-KLC2, whereas abrogation of the WE motif had no obvious effects. A double mutant with both motifs disrupted displayed HA-KLC2 binding similar to the single WD mutant. Thus, the WE motif has a very low affinity for KLC2. Indeed, a 10-amino-acid-long peptide centered on the WD motif (SKIP^{WD}) (Fig. 1A) bound to KLC2^{TPR} with a dissociation constant (K_d) of 24 μ M, whereas the affinity of the equivalent SKIP^{WE} peptide was above 110 μ M (Fig. 1C). The presence of both motifs improves the affinity for KLC2^{TPR} because a 40-amino-acid-long peptide SKIP^{WDWE} that encompasses the W-acid pair sequence bound with an apparent affinity higher than that of the single SKIP^{WD} motif (K_d = 4.9 μ M). Binding affinity measurements at varying NaCl concentrations showed that electrostatic interactions play an important role in the recognition process (fig. S1).

For structural studies, we focused on the SKIP^{WD} motif, and to facilitate the crystallization process, we engineered a chimeric construct in which the SKIP^{WD} peptide was fused N-terminal to KLC2^{TPR} via a flexible (TGS)₄ linker (18). Crystals of SKIP^{WD}-KLC2^{TPR} were grown by using vapor diffusion techniques and diffracted at 2.9 Å by using synchrotron radiation. The final model of the cargo complex is characterized by R and R_{free} values of 20.3 and 24.5%, respectively (table S1). KLC2^{TPR} consists of six TPR repeats (TPR1 to TPR6), each contributed by a classical helix-turn-helix structural motif arranged in a right-handed super-helical conformation, with a non-TPR helix

Randall Division of Cell and Molecular Biophysics, King's College London, London SE1 1UL, UK.

*These authors contributed equally to this work.

†Corresponding authors. E-mail: mark.dodding@kcl.ac.uk (M.P.D.); roberto.steiner@kcl.ac.uk (R.A.S.)

positioned between TPR5 and TPR6 (14). The structure of SKIP^{WD}-KLC2^{TPR} revealed the cargo peptide bound in an extended conformation at the

N-terminal portion of the KLC2^{TPR} concave surface, with a direction parallel to the external helix of the repeat (Fig. 2, A and B, and fig. S2). Al-

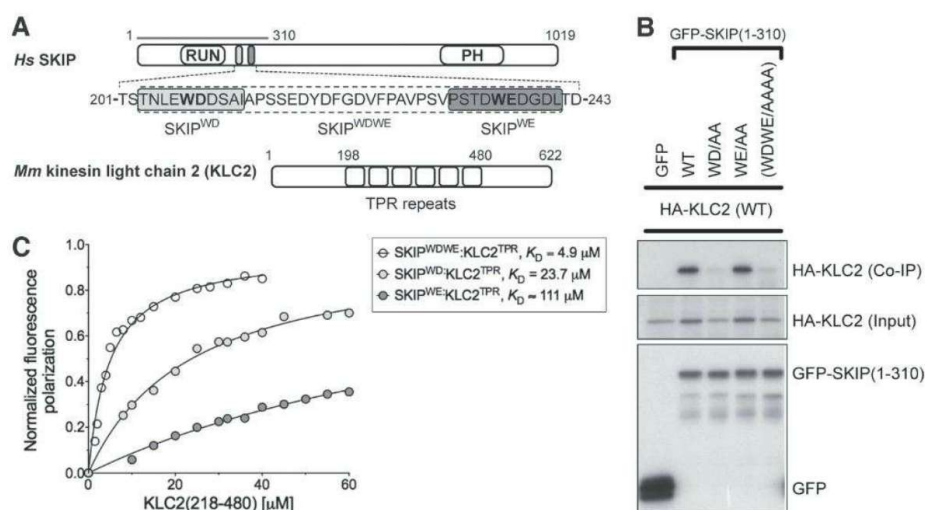


Fig. 1. Binding of SKIP to KLC2. (A) Scheme of SKIP and KLC2. The two W-acidic motifs and KLC2 TPR repeats are highlighted. (B) Coimmunoprecipitation shows that alanine replacement in the first W-acidic motif (WD) abrogates SKIP:KLC2 binding, whereas the same substitution in the second motif (WE) has virtually no effect. (C) Fluorescence polarization measurements confirm that the first SKIP W-acidic motif (SKIP^{WD}) has a higher affinity for the TPR domain of KLC2 than that of the second one (SKIP^{WE}). A peptide encompassing both motifs (SKIP^{WDWE}) binds with higher affinity than the best single motif. K_d values reported here were calculated at 150 mM NaCl. Binding affinity values at varying ionic strength values are given in fig. S1.

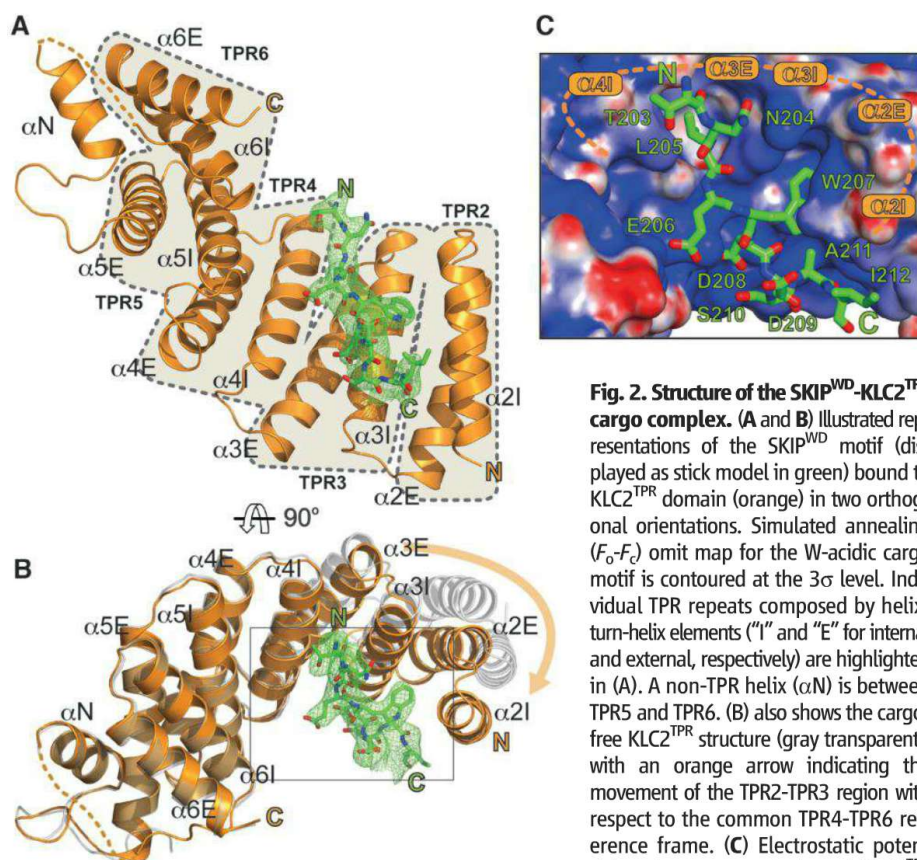


Fig. 2. Structure of the SKIP^{WD}-KLC2^{TPR} cargo complex. (A and B) Illustrated representations of the SKIP^{WD} motif (displayed as stick model in green) bound to KLC2^{TPR} domain (orange) in two orthogonal orientations. Simulated annealing ($F_o - F_c$) omit map for the W-acidic cargo motif is contoured at the 3 σ level. Individual TPR repeats composed by helix-turn-helix elements ("I" and "E" for internal and external, respectively) are highlighted in (A). A non-TPR helix (α N) is between TPR5 and TPR6. (B) also shows the cargo-free KLC2^{TPR} structure (gray transparent), with an orange arrow indicating the movement of the TPR2-TPR3 region with respect to the common TPR4-TPR6 reference frame. (C) Electrostatic potential surface representation of KLC2^{TPR}

though our construct includes the external helix of TPR1, this region, as well as other flexible stretches, could not be unambiguously interpreted in the electron density. Thus, KLC2^{TPR} starts from TPR2 in our model.

A comparison between cargo-free KLC2^{TPR} (3CEQ) and cargo-bound KLC2^{TPR} revealed structural differences considered to arise from a rigid jaw movement of the N-terminal TPR2-3 region, which closes upon cargo recognition engendering the binding surface and pockets for the SKIP^{WD} peptide. (Fig. 2B and fig. S3). An analysis performed by using the Protein Interfaces, Surfaces and Assemblies (PISA) algorithm (19) indicates that all residues of the SKIP^{WD} peptide are involved in formation of the complex, which is stabilized by residues from TPR2-TPR3 and the internal helix of TPR4 (Fig. 2C). This concave groove surface displays a positive electrostatic charge ideally poised to complement the negatively charged W-acidic cargo motifs (Fig. 2C and fig. S4). Overall, the interface area of the SKIP^{WD}-KLC2^{TPR} complex is ~ 770 Å², stabilized by a mixture of H-bonds, salt bridges, and hydrophobic interactions (Fig. 3A).

The W residue central to the motif is generally flanked by amino acids bearing a carboxylate side chain. SKIP^{W207} (position 0, p_0) is buried within a leucine-rich pocket positioned roughly in the middle along the TPR length and contributed by side chains from TPR2 (L248, R251, and L263) and TPR3 (N287, L290, and L291). In particular, the side-chain of N287 is in a conformation so that it serves the dual purpose of stabilizing SKIP^{W207} indole group by lining one side of the pocket while engaging at the same time in hydrogen bonds with the main chain amide and carbonyl oxygen of the residue at $p+1$. The latter position is almost invariably occupied by a glutamic or aspartic acid residue (7). The carboxylate side-chain of SKIP^{D208} points in the opposite direction to that of SKIP^{W207}, engaging in a network of salt bridges and H-bonds with the positively charged R312 and K325 side chains of α 3E and α 4I, respectively. An acidic side chain at position $p-1$ (SKIP^{E206}) of the motif is also very conserved in other W-acidic motifs. Like SKIP^{D208}, the carboxylate side-chain of SKIP^{E206} faces the TPR3-TPR4 side of the KLC2^{TPR} recognition groove, where it is stabilized by an ionic interaction with K325. Position $p-2$ of SKIP^{WD} features a leucine residue (SKIP^{L205}). The hydrophobic side chain of SKIP^{L205} is deeply buried in a hydrophobic pocket formed by residues from TPR3 and TPR4. The side chain of N329 plays a similar dual role as that of N287. Although lining the SKIP^{L205} pocket, it also H-bonds with the main chain at position $p-1$. Together, N287 and N329 act like a clamp on opposite sides of SKIP^{WD}, providing 4 of the 10 hydrogen bonds that stabilize the complex. Residues at positions $p-(3,4)$ are less important for complex stability consistent with their general lack of sequence conservation amongst W-acidic motif cargo (7). The C-terminal stretch of SKIP^{WD} encompassing $p+(2,3,4,5)$

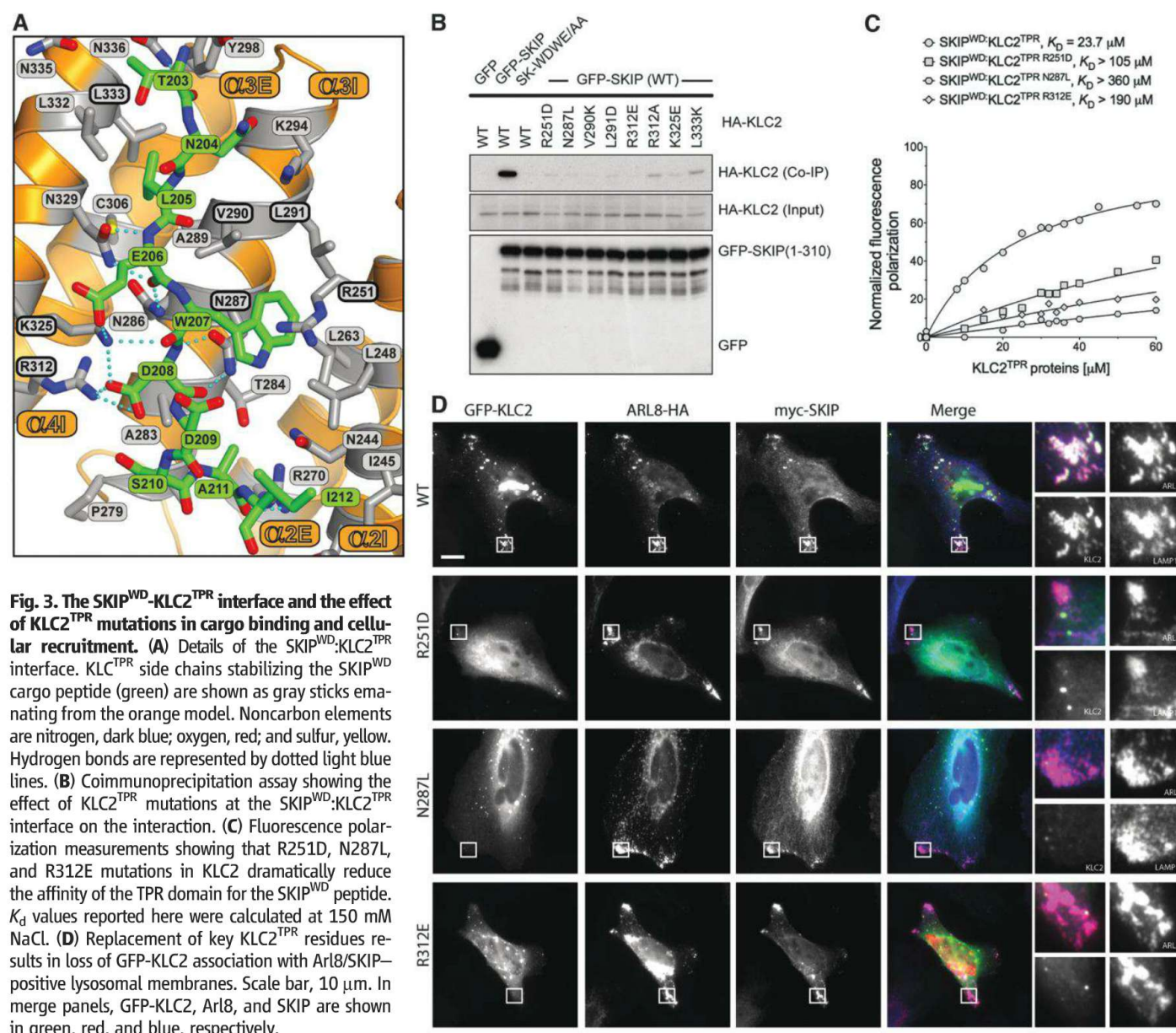
is observed in a more compact, turn-like conformation, with SKIP^{D209,S210} at $p+(2,3)$ making very minor contacts with the groove. As for the N-terminal peripheral region of SKIP^{WD}, the exact nature of the amino acids at $p+(4,5)$ does not seem critical for complex stability. Alternative side chains can be positioned at this topological position, possibly involving a rearrangement of the main chain.

To investigate the effect of amino acid replacements within the KLC2^{TPR} region on the interaction with SKIP, we used immunoprecipitation as described above. All amino acid substitutions tested resulted in abrogation or near-abrogation of complex formation between KLC2 and SKIP (Fig. 3, B and C). The importance of electrostatic interactions in cargo recognition is underscored by charge-reversal mutations (Fig. 3, B and C). When coexpressed in cells, SKIP and its small guano-

sine triphosphatase-binding partner Arl8 associate strongly with lysosomes and promote their trafficking to the cell periphery. GFP-KLC2 associates with the same lysosomes (Fig. 3D and fig. S5) (13). This is essentially abolished by KLC2 mutations (R251D, N287L, and R312E) (Fig. 3D and fig. S5). The same mutations strongly inhibited the binding of KLC2^{TPR} to the WD motif peptide (Fig. 3C). We conclude that optimal SKIP:KLC2 stability critically depends on a very conserved cargo recognition-interaction groove. We extended our analysis to the well-characterized Calsyntenin-1 (CSTN-1) cargo, which also exhibits two W-acidic motifs (9, 11, 12). Differently from SKIP, both CSTN-1 motifs bound to KLC2^{TPR} with similar affinity (fig. S6). However, the mutations in the KLC2-binding groove that disrupted SKIP^{WD} binding also abrogated recognition of CSTN-1 (fig. S6). A sequence alignment shows

total conservation of the KLC2^{TPR} residues interacting with the cargo across the kinesin light chain family (fig. S7). Thus, the concave groove within the TPR domain where the SKIP^{WD} peptide binds is likely to be the primary site of interaction for W-acidic cargo motifs in general. However, we cannot exclude that secondary sites also exist.

In the context of intracellular transport in which kinesin-1 functions as a tetramer containing two KLCs held together by a coiled-coil region and both SKIP motifs contribute to transport (7, 13), it is tempting to speculate that both chains can contribute to the binding of the W-acidic cargo pair. For SKIP, the higher-affinity WD motif would direct the first binding event to one of the KLC^{TPR}, with avidity effect promoting the association of the WE motif to the other KLC (20, 21). It will be important to determine this



relationship to the kinesin-1 tetramer and examine the importance of cargo-induced TPR conformational change in motor activation.

References and Notes

1. N. Hirokawa, Y. Noda, Y. Tanaka, S. Niwa, *Nat. Rev. Mol. Cell Biol.* **10**, 682 (2009).
2. M. P. Dodding, M. Way, *EMBO J.* **30**, 3527 (2011).
3. E. Boucrot, T. Henry, J. P. Borg, J. P. Gorvel, S. Mésès, *Science* **308**, 1174 (2005).
4. J. G. Gindhart, *Brief. Funct. Genomics Proteomics* **5**, 74 (2006).
5. L. D. D'Andrea, L. Regan, *Trends Biochem. Sci.* **28**, 655 (2003).
6. J. W. Hammond, K. Griffin, G. T. Jih, J. Stuckey, K. J. Verhey, *Traffic* **9**, 725 (2008).
7. M. P. Dodding, R. Mitter, A. C. Humphries, M. Way, *EMBO J.* **30**, 4523 (2011).
8. T. Aoyama et al., *J. Cell Sci.* **122**, 4177 (2009).
9. A. Konecna et al., *Mol. Biol. Cell* **17**, 3651 (2006).
10. M. R. Schmidt et al., *Proc. Natl. Acad. Sci. U.S.A.* **106**, 15344 (2009).
11. Y. Araki et al., *EMBO J.* **26**, 1475 (2007).
12. T. Kawano et al., *Traffic* **13**, 834 (2012).
13. C. Rosa-Ferreira, S. Munro, *Dev. Cell* **21**, 1171 (2011).
14. H. Zhu et al., *PLoS ONE* **7**, e33943 (2012).
15. J. R. McGuire, J. Rong, S. H. Li, X. J. Li, *J. Biol. Chem.* **281**, 3552 (2006).
16. L. A. Ligon, M. Tokito, J. M. Finklestein, F. E. Grossman, E. L. Holzbaur, *J. Biol. Chem.* **279**, 19201 (2004).
17. G. W. Morgan et al., *PLoS Pathog.* **6**, e1000785 (2010).
18. L. Pellegrini et al., *Nature* **420**, 287 (2002).
19. E. Krissinel, K. Henrick, *J. Mol. Biol.* **372**, 774 (2007).
20. L. Radnai et al., *J. Biol. Chem.* **285**, 38649 (2010).
21. E. T. Mack et al., *J. Am. Chem. Soc.* **134**, 333 (2012).

Acknowledgments: Scientists of I24 beamline (Diamond Light Source, Didcot, UK) are gratefully acknowledged for their support during data collection. ARL8b-HA and myc-SKIP were a kind gift from S. Munro, (Laboratory of Molecular

Biology—Medical Research Council, Cambridge). We thank T. Blundell and R. Nookala (University of Cambridge) for useful discussions. Coordinates and structure factor files for have been deposited in the Protein Data Bank under accession number 3ZFW. The authors declare no competing financial interests. M.P.D. and A.L. are supported by a Wellcome Trust Research Career Development Fellowship to M.P.D. and a London Law Trust Medal Fellowship to M.P.D. S.P. is supported by a British Heart Foundation grant awarded to R.A.S.

Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1234264/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S7
Table S1
References (22–33)

19 December 2012; accepted 7 March 2013

Published online 21 March 2013;
10.1126/science.1234264

Actin-Propelled Invasive Membrane Protrusions Promote Fusogenic Protein Engagement During Cell-Cell Fusion

Khurts Shilagardi, Shuo Li, Fengbao Luo, Faiz Marikar, Rui Duan, Peng Jin, Ji Hoon Kim, Katherine Murnen, Elizabeth H. Chen*

Cell-cell fusion is critical for the conception, development, and physiology of multicellular organisms. Although cellular fusogenic proteins and the actin cytoskeleton are implicated in cell-cell fusion, it remains unclear whether and how they coordinate to promote plasma membrane fusion. We reconstituted a high-efficiency, inducible cell fusion culture system in the normally nonfusing *Drosophila* S2R+ cells. Both fusogenic proteins and actin cytoskeletal rearrangements were necessary for cell fusion, and in combination they were sufficient to impart fusion competence. Localized actin polymerization triggered by specific cell-cell or cell-matrix adhesion molecules propelled invasive cell membrane protrusions, which in turn promoted fusogenic protein engagement and plasma membrane fusion. This de novo cell fusion culture system reveals a general role for actin-propelled invasive membrane protrusions in driving fusogenic protein engagement during cell-cell fusion.

Cell-cell fusion occurs in many biological processes such as fertilization, myogenesis, placenta formation, bone remodeling, and immune response (1–3). Transmembrane fusogenic proteins are implicated in the fusion of multiple cell types in *Caenorhabditis elegans* (4), whereas actin polymerization is implicated in the fusion of muscle cells in *Drosophila*, zebrafish, and mice (5–7). It remains unknown whether fusogenic proteins and the actin cytoskeleton coordinate during cell-cell fusion, and if so, how this is accomplished. We addressed these questions by reconstituting cell fusion de novo in the otherwise nonfusing S2R+ cells, a hemocyte-like cell line derived from *Drosophila* embryos (8).

Transfection of known components of *Drosophila* myoblast fusion, including cell adhesion molecules (9, 10) and actin cytoskeletal regulators (11–14), failed to induce S2R+ cell fusion,

despite causing extensive cell adhesion and F-actin enrichment at cell-cell contact sites (fig. S1, A to C). Expression of the *C. elegans* fusogenic protein Eff-1 (15, 16) induced low-level S2R+ cell fusion (Fig. 1, A and F). Multinucleate syncytia were observed 24 hours after Eff-1 transfection, and by 72 hours after transfection, ~12% (12.1 ± 1.1%) of Eff-1-positive cells were in multinucleate syncytia, with each syncytium containing a median number of eight nuclei (Fig. 1, F and G). These Eff-1-induced multinucleate syncytia resulted from cell fusion (fig. S2, A to B''), and Eff-1 was required in both fusion partners (fig. S2C), similar to findings reported for moth Sf9 cells (16).

Because close membrane apposition is a prerequisite for membrane fusion, we asked whether Eff-1-induced fusion could be enhanced by co-expressing cell adhesion molecules. Dumbfounded (Duf) and Sticks and stones (Sns) are immunoglobulin (Ig) domain-containing transmembrane proteins that are required for *Drosophila* myoblast fusion (9, 10) but are not normally expressed in S2R+ cells (fig. S1D). Exogenous Duf, but not

Sns, promotes homophilic cell adhesion in cultured *Drosophila* cells (17–19), as does Echinoid (Ed), an Ig domain-containing transmembrane protein not implicated in myoblast fusion (20, 21). Among the three proteins, only Sns enhanced Eff-1-mediated fusion (Fig. 1, B, C, D, and F), which suggests that membrane apposition mediated by cell adhesion per se is not sufficient to promote Eff-1-mediated fusion. Nearly 90% (86.3 ± 2.9%) of the Sns-Eff-1-coexpressing cells were in multinucleate syncytia (Fig. 1C), representing a factor of 7 increase over Eff-1-induced fusion (Fig. 1F). These large syncytia contained up to 220 nuclei, with a median number of 44 nuclei per cell (Fig. 1G). Live imaging confirmed that Sns-Eff-1-induced syncytial formation resulted from cell fusion (fig. S3, A and B, and movies S1 and S2). Besides Sns, overexpression of an α subunit (α PS2) of the cell-matrix adhesion molecule integrin (22), which has been implicated in multiple types of cell fusion events (23–26), enhanced Eff-1-mediated fusion by a factor of 5 (63.9 ± 4.3%) with a median number of 20 nuclei per cell (Fig. 1, E to G). The marked enhancement of Eff-1-mediated cell fusion by Sns and integrin, neither of which facilitated homophilic cell adhesion nor interacted with Eff-1 more strongly than did Duf (fig. S4), prompted us to examine the cellular mechanisms underlying their fusion-enhancing activity.

In *Drosophila*, Sns and Duf trigger distinct actin cytoskeletal changes during myoblast fusion. Sns organizes an F-actin-enriched invasive podosome-like structure (PLS) in the fusion competent myoblast (27, 28), whereas Duf promotes the formation of a thin sheath of actin underlying the apposing founder cell membrane (27). Their differential activity in remodeling the actin cytoskeleton was recapitulated in S2R+ cells, as F-actin-enriched foci were observed at cell-cell contact sites marked by the accumulation of Eff-1 and the cell adhesion molecule in Sns-Eff-1-expressing cells (Fig. 1H and fig. S5A) but not in Duf-Eff-1-expressing cells (Fig. 1I and fig. S5B). Live imaging of Sns-Eff-1-expressing cells using green fluorescent protein (GFP) fused to the F-actin-

Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

*Corresponding author. E-mail: echen@jhmi.edu

binding C-terminal tail of moesin (GFP-moesin) (29) revealed that these F-actin foci corresponded to sites of fusion (Fig. 2A, fig. S6, A and B, and movies S3 to S5). Using a cell-mixing fusion assay, we showed that Sns was required in only one of the two fusion partners to promote efficient cell fusion (fig. S7).

Because the actin nucleation-promoting factors (NPFs) of the Arp2/3 complex, WASP (11–13) and Scar/WAVE (14), are required for Sns-induced PLS formation in *Drosophila* myoblast fusion (27), we investigated whether WASP and Scar are required for Sns-Eff-1-induced cell fusion. RNA interference (RNAi) knockdown of WASP, its binding partner WASP-interacting protein (WIP) (11, 12), or Scar abolished Sns-induced F-actin foci (fig. S8) and eliminated Sns-enhanced cell fusion (Fig. 2B). At sites of fusion, fluorescence recovery after photobleaching (FRAP) analysis revealed more dynamic exchanges of WASP and Scar than of Sns (Fig. 2, C and D, fig. S9, and movies S6 and S7), which suggests that Sns provides a relatively stable organizing center at these sites to recruit WASP and Scar. These findings indicate that dynamic actin cytoskeletal rearrangement is required for Sns-Eff-1-induced cell fusion. RNAi knockdown of the P40 subunit of the Arp2/3 complex in moth Sf9 cells also decreased Eff-1-induced fusion ($7.2 \pm 1.2\%$ versus $16.7 \pm 6.1\%$; fig. S10), indicating that Arp2/3-

mediated actin polymerization is generally required for fusion in different cell types.

To examine whether Arp2/3-mediated actin polymerization is sufficient to enhance Eff-1-mediated fusion, we fused WIP, WASP, or Scar to the C terminus of Duf or Sns and coexpressed each chimeric protein with Eff-1 in S2R+ cells. Attaching WIP to Sns did not affect Sns's ability to organize actin polymerization at cell-cell contact sites (fig. S11A) or to enhance fusion (Fig. 2E). Attaching WIP to Duf induced the formation of F-actin-enriched hairlike protrusions at cell-cell contact sites (fig. S11B) and converted Duf into a fusion-promoting molecule (Fig. 2F). This result suggests that WIP-mediated actin cytoskeletal rearrangement is sufficient to enhance Eff-1-mediated cell fusion. Unexpectedly, attaching WASP or Scar directly to Duf did not enhance Eff-1-mediated fusion (Fig. 2F), and attaching these NPFs to Sns abolished Sns's fusion-enhancing activity (Fig. 2E) because of mislocalization of the chimeric proteins. Unlike Duf-WIP or Sns-WIP, which were correctly targeted to the cell membrane (fig. S11, A and B), Duf-WASP, Duf-Scar, Sns-WASP, and Sns-Scar were localized in the cytoplasm (fig. S11, C and D), where they induced actin comet tails propelling rapid movement of vesicles containing Eff-1 and the corresponding chimeric protein (fig. S11, C and D, and movies S8 and S9). Thus, localized Arp2/3-mediated actin

polymerization at cell-cell contact sites promotes Eff-1-mediated fusion.

Because Arp2/3 nucleates a branched actin network that drives membrane protrusions, we predicted that the F-actin foci at cell-cell contact sites may be invasive. To test this, we conducted super-resolution total internal reflection fluorescence (TIRF)-based stochastic optical reconstruction microscopy (STORM) (30) and ultrastructural electron microscopy (EM). Both analyses revealed a tightly packed group of finger-like protrusions extending from one cell into its fusion partner in Sns-Eff-1-expressing cells but not in Duf-Eff-1-expressing cells (Fig. 2, G and H, b and c; Fig. 3, A and A'; and fig. S12), consistent with the reported ultrastructure of the invasive PLS in *Drosophila* myoblast fusion (27, 31, 32). These fingers contained distinct clusters of Sns and Eff-1 (Fig. 2G, d to d') and frequently overlapping Sns and actin signals (Fig. 2H, d to d'). Strikingly, segments of electron-dense "ladders" were present between the apposing membranes along the invasive fingers (Fig. 3, A' and A''). These electron-dense ladders grossly resembled the electron-dense spikes formed by the virus-packaged Eff-1 on the viral envelope (33) (albeit thinner than the latter) and spatially corresponded to clusters of Eff-1 on the invasive fingers observed by STORM (Fig. 2G, d'). Immunogold labeling confirmed the presence of Eff-1 on the membranes

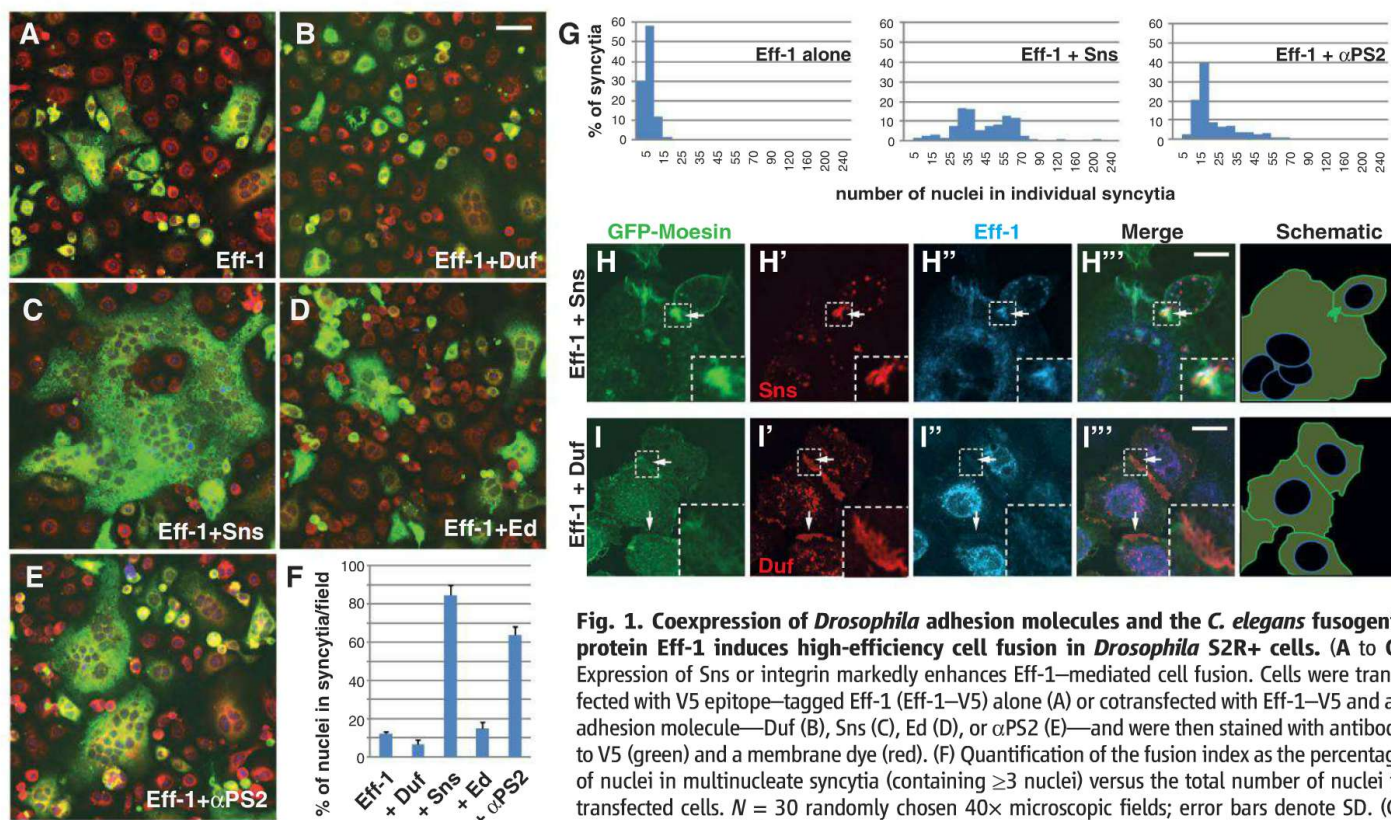


Fig. 1. Coexpression of *Drosophila* adhesion molecules and the *C. elegans* fusogenic protein Eff-1 induces high-efficiency cell fusion in *Drosophila* S2R+ cells. (A to G) Expression of Sns or integrin markedly enhances Eff-1-mediated cell fusion. Cells were transfected with V5 epitope-tagged Eff-1 (Eff-1-V5) alone (A) or cotransfected with Eff-1-V5 and an adhesion molecule—Duf (B), Sns (C), Ed (D), or α PS2 (E)—and were then stained with antibody to V5 (green) and a membrane dye (red). (F) Quantification of the fusion index as the percentage of nuclei in multinucleate syncytia (containing ≥ 3 nuclei) versus the total number of nuclei in transfected cells. $N = 30$ randomly chosen $40\times$ microscopic fields; error bars denote SD. (G) Distribution of nuclei number in multinucleate syncytia. (H and I) Sns and Duf trigger distinct

actin cytoskeletal rearrangement at cell-cell contact sites. Cells were cotransfected with hemagglutinin-tagged Eff-1 (Eff-1-HA), GFP-moesin, and Sns-V5 (H) or Duf-V5 (I), and stained with antibodies to V5 (red) and HA (cyan). F-actin was visualized by GFP-moesin (green). Boxed areas are magnified in insets; schematic drawings of F-actin are shown at the right. Arrow in (H): an F-actin-enriched focus associated with Sns and Eff-1 accumulation at a cell-cell contact site. Arrows in (I): cell-cell contact sites enriched with Duf but not F-actin. Scale bars, $40\ \mu\text{m}$ [(A) to (E)], $10\ \mu\text{m}$ [(H) and (I)].

along the invasive fingers (fig. S13, B and B'). Thus, in Sns-Eff-1-induced cell fusion, Arp2/3-mediated actin polymerization generates fingerlike membrane protrusions that promote Eff-1 engagement across the apposing cell membranes.

To determine whether actin-propelled membrane protrusions are generally involved in cell-cell fusion, we investigated how integrin modulated Eff-1-mediated fusion. Loss or gain of α PS2 function abolished or enhanced Eff-1-mediated cell fusion commensurate with the strength of

cell-matrix adhesion (Fig. 1, E and F, and Fig. 4, A to C). Unlike Sns-Eff-1-induced fusion, α PS2-Eff-1-induced fusion only required Scar but not the WASP-WIP complex (Fig. 4A and fig. S14A), the latter of which is normally recruited to sites of myoblast fusion by Sns in *Drosophila* (11, 12). As a consequence, α PS2-Eff-1-expressing cells formed numerous F-actin-containing hairlike projections, instead of dense F-actin foci, along the broad cell-cell contact zone (Fig. 4D and movie S10). Notably, cells expressing Eff-1 alone also

occasionally formed multiple hairlike projections along cell-cell contact zones (Fig. 4E), and RNAi of Scar, but not of WASP or WIP, abolished the basal level of Eff-1-mediated cell fusion (Fig. 4B and fig. S14B). STORM and EM analyses revealed individual fingerlike protrusions sparsely localized at the cell periphery and along the cell-cell contact zone of α PS2-Eff-1-expressing cells (Fig. 3B and Fig. 4, F and G, b and c), corresponding to the hairlike projections visualized by confocal microscopy (Fig. 4D).

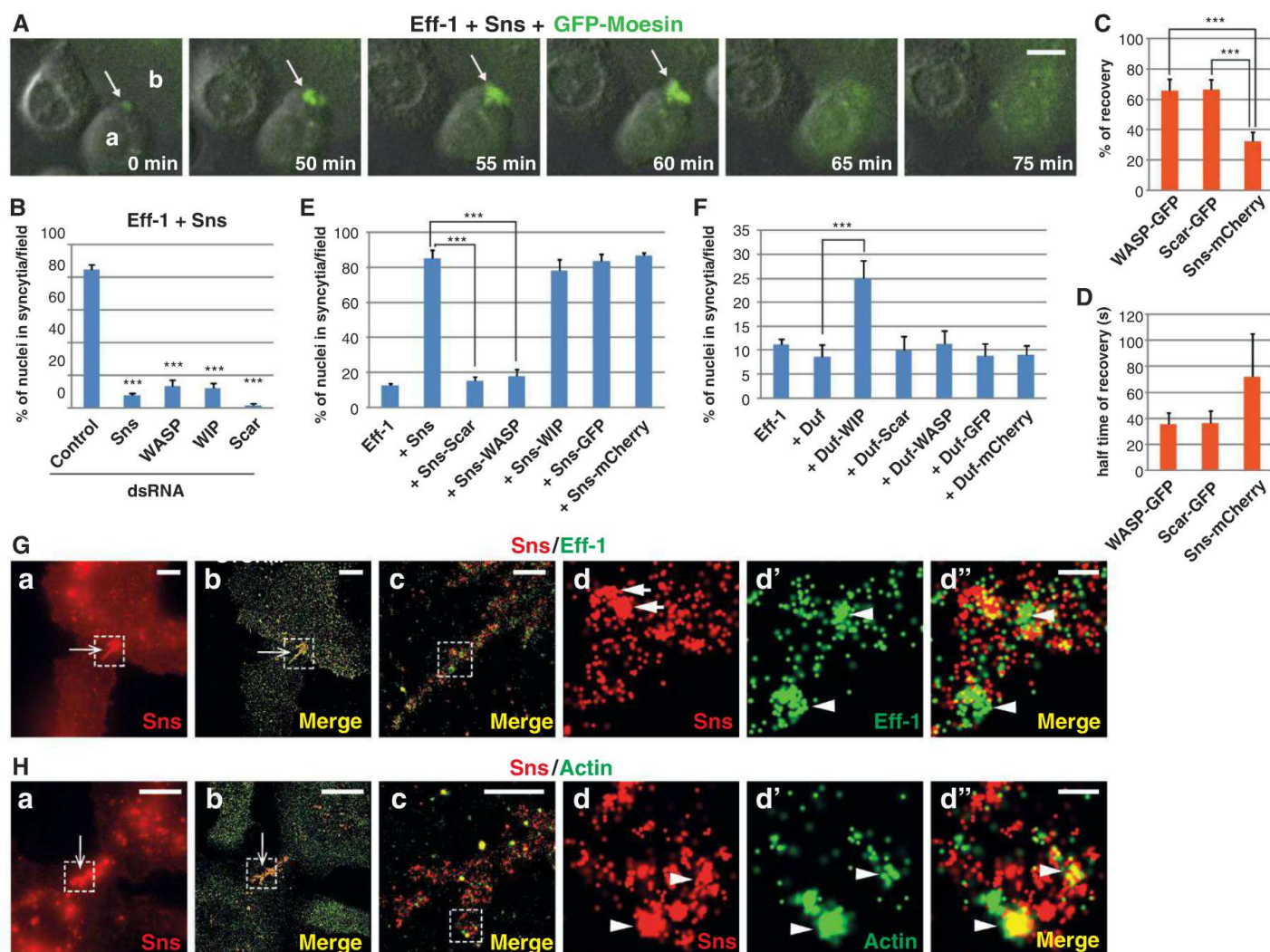
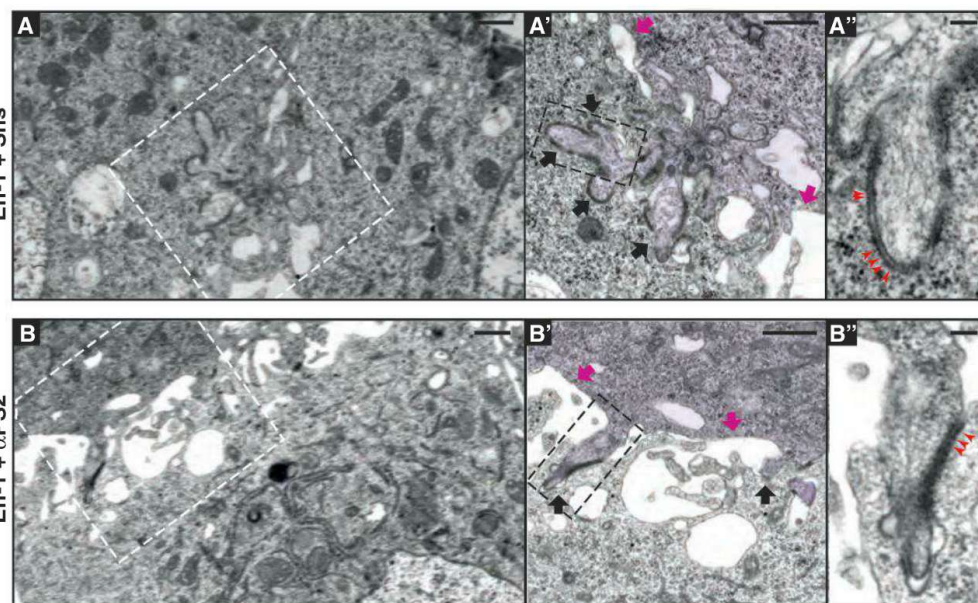


Fig. 2. Actin-propelled invasive fingerlike protrusions promote high-efficiency cell fusion in Sns-Eff-1-expressing S2R+ cells. (A) A dynamic F-actin focus mediating cell fusion. Cells coexpressing Eff-1, Sns, and GFP-moesin were subjected to time-lapse imaging. Stills from representative movie S3 are shown; 0 min corresponds to 3:00:51 in movie S3. Arrow indicates the F-actin focus at the protruding tip of cell a, which was about to fuse with cell b. Scale bar, 10 μ m. (B) Arp2/3-mediated actin polymerization is required for Sns-enhanced cell fusion. Data are fusion indices ($\pm$ SD) resulting from RNAi knockdown of Sns, WASP, WIP, or Scar in cells coexpressing Sns and Eff-1 treated with corresponding double-stranded RNA (dsRNA). Statistical significance was determined using the two-tailed Student *t* test (***) $P < 0.001$ by comparing control and RNAi samples. (C and D) FRAP assays of WASP, Scar, and Sns at sites of fusion. Sns-mCherry and WASP-GFP (or Scar-GFP) foci were photobleached and monitored with respect to recovery percentage (C) and recovery half-time (D) in cells coexpressing Eff-1, Sns-mCherry, and

WASP-GFP (or Scar-GFP). $N = 4$; error bars denote SD. (E and F) Effects of chimeric proteins of cell adhesion molecules and Arp2/3 regulators on cell fusion. Cells were transfected with Eff-1 alone or cotransfected with Eff-1 and indicated plasmids. Fusion indices ($\pm$ SD) were quantified as described. (G and H) Sns and Eff-1 accumulate in distinct clusters on the cell membrane along the fingerlike protrusions. Cells cotransfected with Sns-V5 and Eff-1-HA were stained with antibodies to V5 (red), HA [green in (G)], and actin [green in (H)], and subjected to STORM analysis. Images were acquired by either wide-field microscopy (a) or STORM [(b) to (d'')]. Boxed area in (a) and (b) is enlarged in (c); boxed area in (c) is enlarged in (d) to (d''). Arrow in (a) and (b) indicates fingerlike protrusions extending from a cell to its fusion partner. In (G), distinct clusters of Sns and Eff-1 are indicated by arrows in (d) and arrowheads in (d') and (d''), respectively. In (H), arrowheads in (d) to (d'') indicate the frequently overlapping signals of Sns and actin. Scale bars, 20 μ m [(a) and (b)], 500 nm (c), 100 nm [(d) to (d'')].

Fig. 3. Invasive fingerlike membrane protrusions promote fusogenic protein engagement.

(A) Electron micrographs of Sns-Eff-1-expressing cells. Boxed area in (A) is enlarged in (A'); boxed area in (A') is enlarged in (A''). (A) Low-magnification view of two adherent cells. (A') Cell on the right (pseudo-colored purple) extended a group of fingerlike protrusions (black arrows) to invade the cell on the left. Segments of electron-dense materials were present on the membranes along the protrusive fingers but were absent elsewhere on the cell membrane (magenta arrows). (A'') At higher magnification, the electron-dense materials showed a ladderlike pattern between the two apposing cell membranes (red arrowheads). (B) Electron micrographs of α PS2-Eff-1-expressing cells. Boxed area in (B) is enlarged in (B'); boxed area in (B') is enlarged in (B''). (B) Low-magnification view of two adherent cells. (B') Cell at the top (pseudo-colored purple) extended individual fingerlike protrusions to invade the cell at the bottom. The invasive fingers (black arrows) were scattered along the broad cell-cell contact zone and were associated with segments of electron-dense materials, which were absent elsewhere on the cell membrane (magenta arrows). (B'') At higher



magnification, the electron-dense materials also showed a ladderlike appearance (red arrowheads) between the two apposing membranes, as in Sns-Eff-1-expressing cells (A''). Scale bars, 500 nm [(A), (A'), (B), and (B')], 100 nm [(A'') and (B'')].

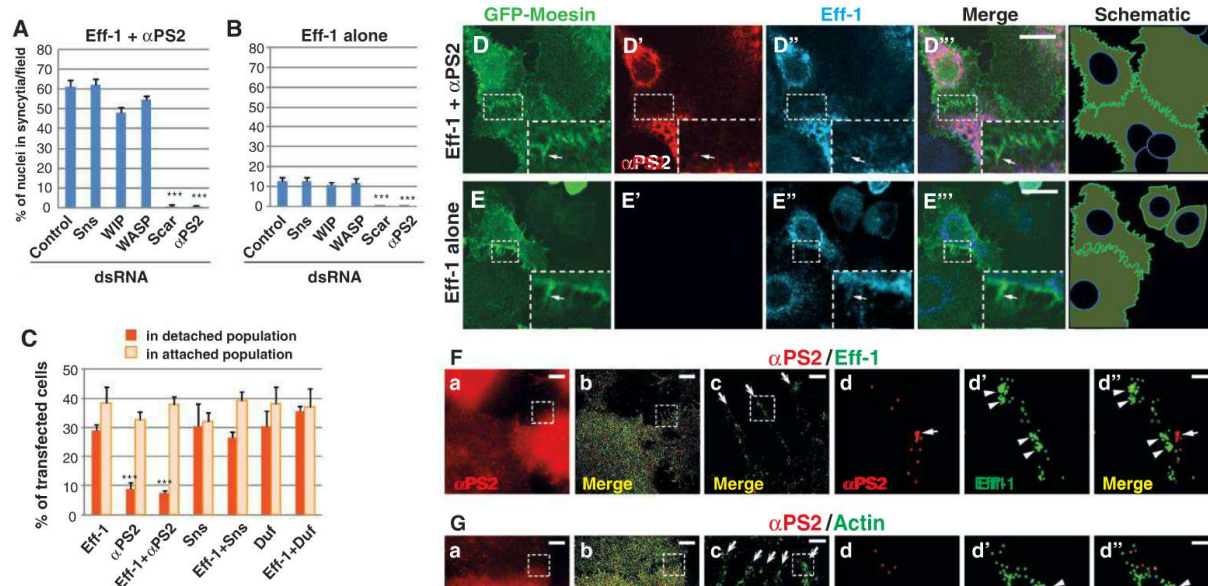


Fig. 4. F-actin-enriched hairlike projections promote cell fusion in α PS2-Eff-1-expressing S2R+ cells.

(A) Scar, but not WASP-WIP, is required for α PS2-Eff-1-induced cell fusion. Fusion indices resulting from RNAi knockdown of Sns, WASP, WIP, Scar, or α PS2 in α PS2-Eff-1-expressing cells are shown. (B) Cell fusion induced by Eff-1 alone is mediated by endogenous Scar and α PS2. Fusion indices resulting from RNAi knockdown of indicated genes in Eff-1-expressing cells are shown. (C) Cells overexpressing α PS2 show stronger adhesion to the culture dish. Cells expressing indicated proteins were subjected to adhesion assays (see supplementary materials). α PS2 expression reduced the percentage of transfected cells in the detached population. Error bars in (A) to (C) denote SD. (D and E) F-actin-enriched hairlike projections along the cell-cell contact zone of α PS2-Eff-1- and Eff-1-expressing cells. Cells were transfected with GFP-moesin and α PS2-Eff-1 (D) or Eff-1 alone (E), and stained with antibodies to V5 (red; α PS2-V5) and HA (cyan; Eff-1-HA). Boxed areas are magnified in insets; schematic drawings of F-actin are at the right. Inset in (D) shows F-actin-enriched

hairlike projections at the cell-cell contact zone of α PS2-Eff-1-expressing cells. Arrow in (D) to (D'') indicates a small punctum of α PS2 within a hairlike projection. Inset in (E) shows the occasionally observed F-actin-enriched projections (one indicated by arrow) in Eff-1-expressing cells. Scale bars, 10 μ m. (F and G) Distinct distribution of α PS2 and Eff-1 on the membrane along the F-actin-enriched fingerlike protrusions. Cells cotransfected with α PS2-V5 and Eff-1-HA were stained with antibodies to V5 (red), HA [green in (F)], and actin [green in (G)] and subjected to STORM analysis. Images were acquired by either wide-field microscopy (a) or STORM [(b) to (d'')]. Boxed area in (a) and (b) is enlarged in (c); boxed area in (c) is enlarged in (d) to (d''). Arrows in (c) indicate individual fingerlike protrusions. Arrow in (d) and (d'') and arrowheads in (d') and (d'') indicate distinct domains of α PS2 and Eff-1 (or actin). Scale bars, 20 μ m [(a) and (b)], 500 nm (c), 100 nm [(d) to (d'')].

These fingerlike protrusions contained Eff-1 clusters on the membrane (Fig. 4F, d and d'') and were anchored at their basal side by exogenous α PS2 (Fig. 4, F and G, d to d''). As in Sns-Eff-1-expressing cells (Fig. 3A''), the invasive fingers in α PS2-Eff-1-expressing cells contained segments of electron-dense ladders (Fig. 3B'') corresponding to the Eff-1 clusters observed by STORM (Fig. 4F, d' and d''). Thus, despite the differences in the requirement of actin regulators (Scar versus Scar-WASP) and the overall morphology of F-actin enrichment (hairs versus foci), α PS2-Eff-1-expressing cells and Sns-Eff-1-expressing cells use similar invasive fingerlike membrane protrusions to promote fusogenic protein engagement during cell fusion.

Our studies reveal two fundamental principles underlying cell-cell fusion. First, a transmembrane fusogenic protein is indispensable for cell-cell fusion, because fusion does not occur without a fusogenic protein, irrespective of actin cytoskeletal remodeling. Second, the actin cytoskeleton provides an active driving force for cell-cell fusion by generating membrane protrusions that are necessary and sufficient to promote fusion mediated by fusogenic proteins. Membrane protrusions induced by different adhesion molecules share common characteristics of invasiveness and engagement of fusogenic proteins and therefore represent a general cell-cell fusion mechanism.

References and Notes

1. E. H. Chen, E. N. Olson, *Science* **308**, 369 (2005).
2. B. M. Ogle, M. Cascalho, J. L. Platt, *Nat. Rev. Mol. Cell Biol.* **6**, 567 (2005).
3. M. Oren-Suissa, B. Podbilewicz, *Trends Cell Biol.* **17**, 537 (2007).
4. O. Avinoam, B. Podbilewicz, *Curr. Top. Membr.* **68**, 209 (2011).
5. K. Rochlin, S. Yu, S. Roy, M. K. Baylies, *Dev. Biol.* **341**, 66 (2010).
6. E. H. Chen, *Curr. Top. Membr.* **68**, 235 (2011).
7. S. M. Abmayr, G. K. Pavlath, *Development* **139**, 641 (2012).
8. S. Yanagawa, J. S. Lee, A. Ishimoto, *J. Biol. Chem.* **273**, 32353 (1998).
9. M. Ruiz-Gómez, N. Coutts, A. Price, M. V. Taylor, M. Bate, *Cell* **102**, 189 (2000).
10. B. A. Bour, M. Chakravarti, J. M. West, S. M. Abmayr, *Genes Dev.* **14**, 1498 (2000).
11. S. Kim *et al.*, *Dev. Cell* **12**, 571 (2007).
12. R. Massarwa, S. Carmon, B. Z. Shilo, E. D. Schejter, *Dev. Cell* **12**, 557 (2007).
13. G. Schäfer *et al.*, *Dev. Biol.* **304**, 664 (2007).
14. B. E. Richardson, K. Beckett, S. J. Nowak, M. K. Baylies, *Development* **134**, 4357 (2007).
15. W. A. Mohler *et al.*, *Dev. Cell* **2**, 355 (2002).
16. B. Podbilewicz *et al.*, *Dev. Cell* **11**, 471 (2006).
17. H. A. Dworak, M. A. Charles, L. B. Pellerano, H. Sink, *Development* **128**, 4265 (2001).
18. B. J. Galletta, M. Chakravarti, R. Banerjee, S. M. Abmayr, *Mech. Dev.* **121**, 1455 (2004).
19. E. H. Chen, B. A. Pryce, J. A. Tzeng, G. A. Gonzalez, E. N. Olson, *Cell* **114**, 751 (2003).
20. R. Islam, S. Y. Wei, W. H. Chiu, M. Hortsch, J. C. Hsu, *Development* **130**, 2051 (2003).
21. C. A. Johnston, K. Hirono, K. E. Prehoda, C. Q. Doe, *Cell* **138**, 1150 (2009).
22. P. J. Gotwals, S. E. Paine-Saunders, K. A. Stark, R. O. Hynes, *Curr. Opin. Cell Biol.* **6**, 734 (1994).

23. M. Schwander *et al.*, *Dev. Cell* **4**, 673 (2003).
24. P. Fénel, M. Durand-Clément, *Hum. Reprod.* **13** (suppl. 4), 31 (1998).
25. A. K. McNally, J. M. Anderson, *Am. J. Pathol.* **160**, 621 (2002).
26. N. Tabata *et al.*, *J. Immunol.* **153**, 3256 (1994).
27. K. L. Sens *et al.*, *J. Cell Biol.* **191**, 1013 (2010).
28. S. Haralalka *et al.*, *Development* **138**, 1551 (2011).
29. K. A. Edwards, M. Demsky, R. A. Montague, N. Weymouth, D. P. Kiehart, *Dev. Biol.* **191**, 103 (1997).
30. M. J. Rust, M. Bates, X. Zhuang, *Nat. Methods* **3**, 793 (2006).
31. P. Jin *et al.*, *Dev. Cell* **20**, 623 (2011).
32. R. Duan *et al.*, *J. Cell Biol.* **199**, 169 (2012).
33. O. Avinoam *et al.*, *Science* **332**, 589 (2011).

Acknowledgments: We thank P. Beachy for the S2R+ cell line and G. Seydoux for the *C. elegans* cDNA; M. Delannoy at Johns Hopkins Microscope Facility for advice on cell culture TEM and immunoEM; J. Reidler, J. Hill, and J. DeWitt at Nikon Instruments Inc. for advice on STORM; and S. Craig, J. Nathans, E. Olson, D. Pan, D. Robinson, G. Seydoux, J. Yang, and members of the Chen lab for discussions and critical reading of the manuscript. K.S. was a postdoctoral fellow of the American Heart Association. Supported by the National Institutes of Health (R01 GM098816) and the Packard Foundation (E.H.C.).

Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1234781/DC1
Materials and Methods
Figs. S1 to S14
Table S1
Movies S1 to S10
References (34–36)

4 January 2013; accepted 22 February 2013
Published online 7 March 2013;
10.1126/science.1234781

Bat and Rat Neurons Differ in Theta-Frequency Resonance Despite Similar Coding of Space

James G. Heys,^{1*} Katrina M. MacLeod,² Cynthia F. Moss,³ Michael E. Hasselmo^{4*}

Both bats and rats exhibit grid cells in medial entorhinal cortex that fire as they visit a regular array of spatial locations. In rats, grid-cell firing field properties correlate with theta-frequency rhythmicity of spiking and membrane-potential resonance; however, bat grid cells do not exhibit theta rhythmic spiking, generating controversy over the role of theta rhythm. To test whether this discrepancy reflects differences in rhythmicity at a cellular level, we performed whole-cell patch recordings from entorhinal neurons in both species to record theta-frequency resonance. Bat neurons showed no theta-frequency resonance, suggesting grid-cell coding via different mechanisms in bats and rats or lack of theta rhythmic contributions to grid-cell firing in either species.

The medial entorhinal cortex (mEC) of rodents, humans, nonhuman primates, and bats encodes space by using similar neural firing patterns (1–5). Unit recordings from awake-behaving rodents and bats demonstrate that single neurons in the mEC, termed grid cells, fire when an animal traverses spatial locations on a periodic triangular array, tiling the environment. In rodents, grid-cell coding correlates with rhythmicity at theta frequency (4 to 10 Hz). Subthreshold membrane-potential resonance (sMPR) at theta frequency matches the anatomical dis-

tribution of grid cells (6) and differs along the dorsal-to-ventral axis of rodent mEC (7–11), in proportion with grid-cell firing field properties (2). Rodent grid-cell spiking shows precession in theta phase relative to location (12), theta rhythmicity correlated with grid field properties and running velocity (13, 14), and loss of spatial periodicity in the absence of network theta rhythm (15, 16).

However, bat recordings demonstrate grid cells in mEC and place cells in the hippocampus in the absence of continuous theta-frequency

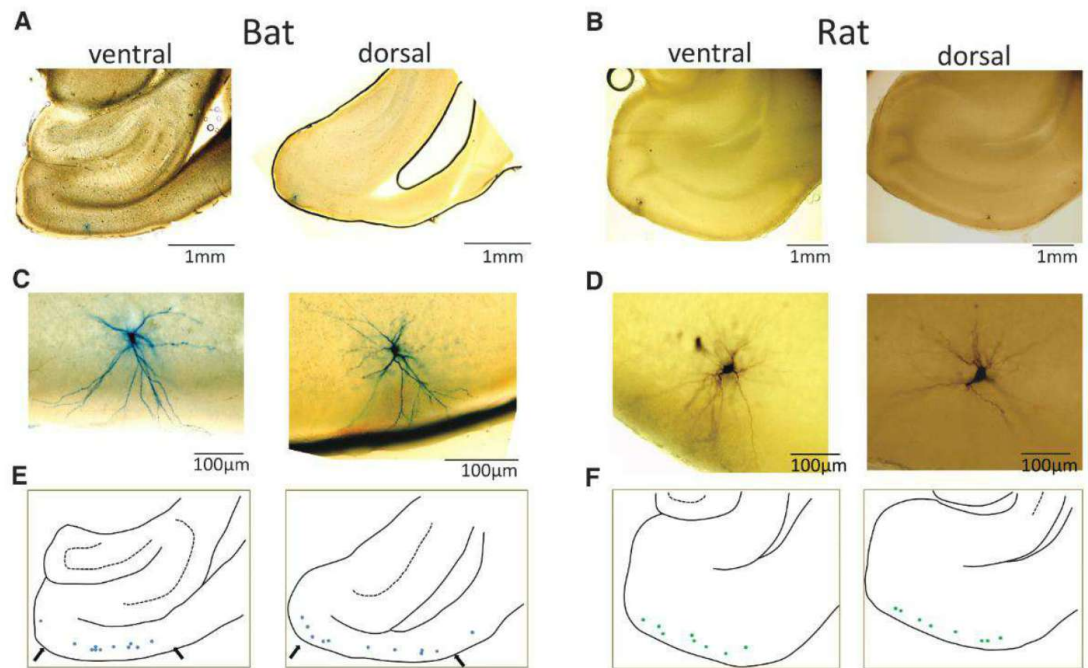
rhythmicity in field potentials or spike-train autocorrelograms (5, 17), arguing against theta-rhythmic mechanisms for grid-cell firing, but controversy remains that theta rhythmicity could be masked by low firing rates in bat grid cells (18). To test for intrinsic theta-rhythmic mechanisms in the bat species *Eptesicus fuscus* and *Rousettus aegyptiacus*, we made in vitro whole-cell patch recordings in brain slices, looking for theta-frequency sMPR. In contrast to layer II stellate cells in rodent mEC, layer II neurons of bat mEC do not show theta-frequency sMPR. These data corroborate earlier bat unit recording data (5, 17) showing that continuous theta rhythmicity during navigation is not present in all mammalian species.

Previous studies demonstrated that parahippocampal areas in bat and rodent brains show similar anatomical organization (5, 19, 20). Neurons loaded with biocytin during whole-cell patch clamp recordings in horizontal brain slices were stained to reveal position and morphology (Fig. 1). Rat neurons were layer II stellate cells on the

¹Graduate Program for Neuroscience, Center for Memory and Brain, Boston University, 2 Cummington Street, Boston, MA 02215, USA. ²Department of Biology, University of Maryland, College Park, MD 20742, USA. ³Department of Psychology, University of Maryland, College Park, MD 20742, USA. ⁴Department of Psychology, Center for Memory and Brain, Boston University, 2 Cummington Street, Boston, MA 02215, USA.

*Corresponding author. E-mail: hasselmo@bu.edu (M.E.H.); jimheys@bu.edu (J.G.H.)

Fig. 1. Anatomy and morphology of layer II mEC neurons in bat and rat. Low-power images of neurons recorded in mECs from bat (A) and rat (B) in ventral (left) and dorsal (right) positions. High-power images show that mEC neurons in bat (C) and rat (D) have stellatelike appearance in ventral (left) and dorsal (right) locations. Recording locations labeled for bat (blue) (E) and rat neurons (green) (F) in ventral (left) and dorsal slices (right). Arrows indicate approximate mEC borders based on anatomical studies (20).



basis of position, morphology, and electrophysiology (21, 22). Bat neurons showed similar patterns of arborization compared to rat stellate cells (Fig. 1, C and D). To ensure that bat recordings included mEC neurons, we sampled a wide range of medial to lateral parahippocampal positions (Fig. 1E and fig. S1).

Recordings from layer II mEC cells with stellate morphology demonstrate prominent electrophysiological differences between the species. Impedance profiles from bat layer II cells do not show the characteristic theta-frequency sMPR present in rat stellate cells (Fig. 2 and fig. S2). Mean resonance frequency measured at a membrane potential of -70 mV in bat neurons was 1.67 ± 0.13 Hz (SE) ($n = 24$), contrasting with theta-band resonance frequency in rat of 8.45 ± 1.19 Hz ($n = 13$) ($P < 0.01$) (Fig. 2E). Mean resonance frequency at -80 mV in bat was 1.77 ± 0.17 Hz ($n = 24$) and 8.86 ± 1.03 Hz ($n = 12$) in rat ($P < 0.01$) (Fig. 2F). Mean resonance strength in bat was 1.10 ± 0.02 at -70 mV (Fig. 2E) and 1.06 ± 0.01 ($n = 24$ cells) at -80 mV (Fig. 2F). In contrast, rat neurons show a band-pass impedance profile with a mean resonance strength of 1.47 ± 0.07 ($n = 13$) ($P < 0.01$) at -70 mV (Fig. 2E) and 1.36 ± 0.04 ($n = 12$ cells) ($P < 0.01$) at -80 mV (Fig. 2F).

Rat stellate cells show a large, inwardly rectifying current during hyperpolarizing input from expression of h current (I_h) (22–24), causing a depolarizing sag in voltage. Bat mEC neurons had significantly smaller sag ratio at multiple membrane potentials [sag ratio at -80 mV for bat was 0.13 ± 0.02 ($n = 26$ cells); for rat, 0.32 ± 0.02 ($n = 14$ cells) ($P < 0.01$)] (Fig. 3B). The sag time constant was significantly slower in bat neurons [sag time constant at -80 mV in bat, $178.88 \pm$

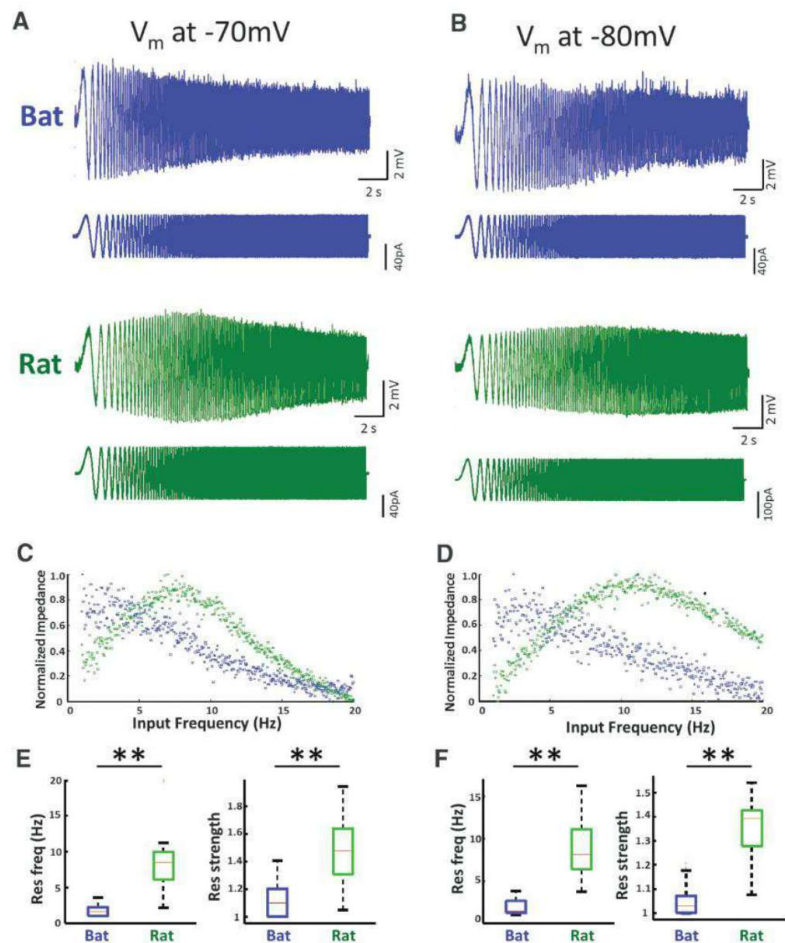


Fig. 2. Lack of theta-frequency resonance in bat neurons. Responses are shown for single bat neurons (blue, top) and single rat stellate cells (green, bottom), measured at membrane potentials (V_m) of -70 (A) and -80 mV (B). Normalized impedance plots from responses shown in (A) and (B) are depicted in (C) and (D). Box plots depict the quartiles of the resonance frequency measured at -70 (E) and -80 mV (F). $**P < 0.01$.

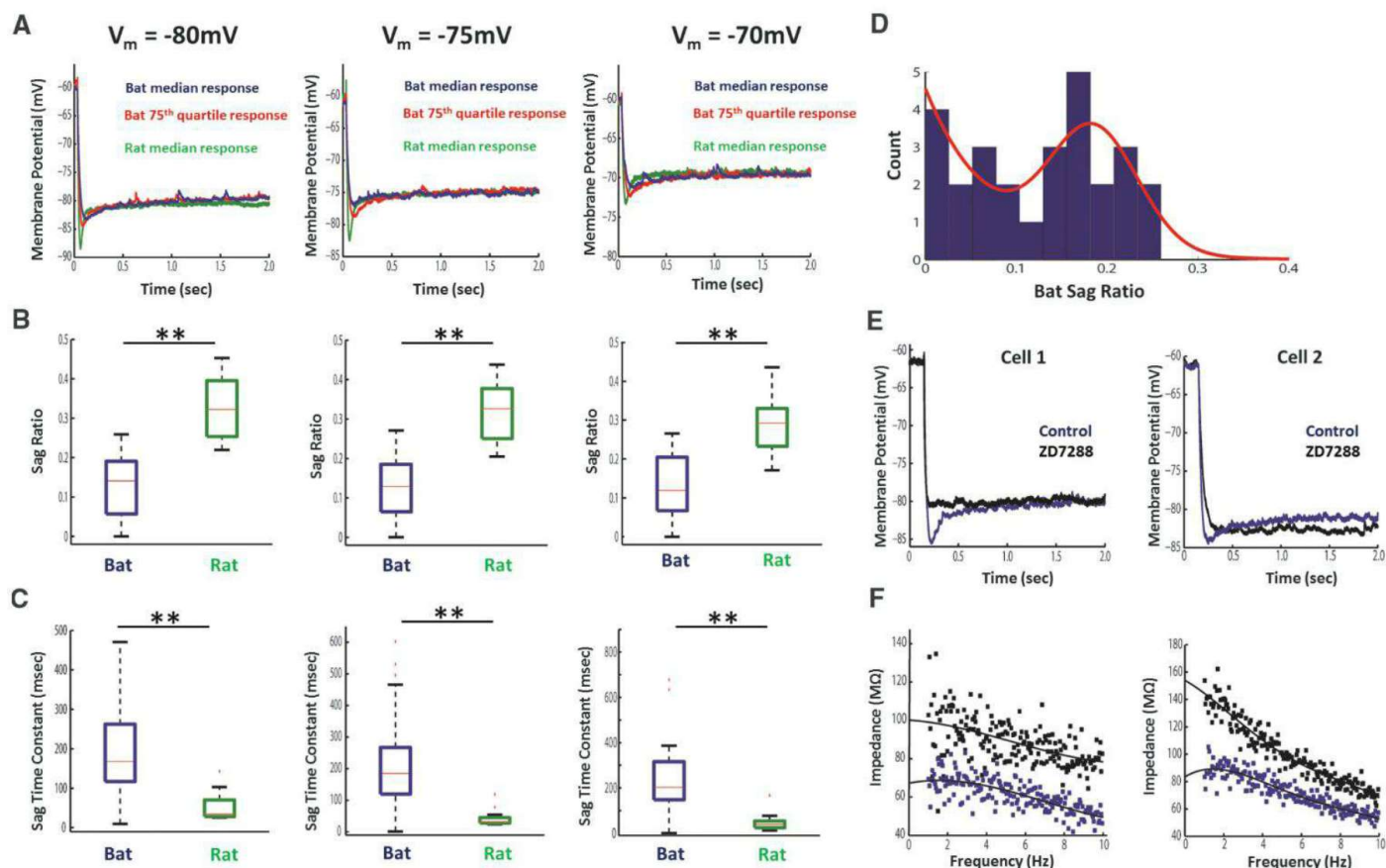


Fig. 3. Membrane potential sag is weaker and slower in bat neurons. (A) Voltage responses measured at steady-state membrane potentials of -80 (left), -75 (middle), and -70 mV (right). Sag ratios (B) and time constants (C) in bat (blue) and rat (green) neurons at -80 (left), -75 (middle), and -70 mV

(right). $**P < 0.01$. (D) A histogram of bat sag ratios at -80 mV appears bimodal. (E) Sag responses in bat cell 1 (left) and cell 2 (right) in control are abolished in 10μ M ZD7288. (F) Impedance profile in control for cell 1 (left) and cell 2 (right) in control and after application of ZD7288.

24.55 ms ($n = 24$); in rat, 53.45 ± 9.85 ms ($n = 14$) ($P < 0.01$) (Fig. 3C). However, inward rectification was still notable in many bat cells. The sag ratio across all bat neurons at -80 mV exhibited a bimodal distribution (Fig. 3D). With a third-order Gaussian fit, the second mode occurred at a sag ratio of 0.181. The 75th quartile sag response reflects the larger sag response in some bat neurons (Fig. 3A). The mean resonance frequency of bat neurons exhibiting the largest quartile sag ratio is still well below theta frequency (2.12 ± 0.32 Hz, $n = 6$). To demonstrate that sag in bat neurons arises from h current, we applied hyperpolarizing current steps in control artificial cerebrospinal fluid and after bath application of 10μ M ZD7288. Sag response (Fig. 3E) and resonance (Fig. 3F) were abolished after blockade of I_h (input current was adjusted for initial voltage of -60 mV and steady-state voltage of -80 mV), consistent with loss of theta frequency sMPR and sag in rat stellate cells during blockade of h current or genetic knockout of HCN1 (8, 10, 24, 25).

Even bat neurons showing sag potentials did not reveal theta-frequency sMPR. To explain this, we built two biophysical conductance models (materials and methods) with morphological and

electrophysiological properties of bat and rat neurons. A simplified model used h-current conductance density (G_h) and time constant (τ_h) based on single exponential fit to the sag response (Fig. 4A). The optimal parameterization combines lower-amplitude G_h (Fig. 4C, left) and slower τ_h (Fig. 4C, right) to match the sag response and non-theta-frequency resonance in bat experiments compared with theta-frequency resonance in rats (Fig. 4E). For rat and bat neurons, 20,000 simulations with different parameters showed that the experimentally derived sag ratio and time constant only occur at a single localized region of parameter space (Fig. 4G). By using a double exponential form of I_h , models simulated both the fast and slow component of the sag response (Fig. 4B). Similar to previous models of rat stellate cells (10, 23, 26), optimal fits gave a fast h current conductance density about 4.5 times larger than slow h current (Fig. 4D). In contrast, best fits for the bat gave a slow h current conductance density 1.3 times larger than the fast conductance (Fig. 4D).

The lack of theta rhythmicity in spike-train autocorrelograms from bat grid cells and place cells (5, 17) has been argued to result from the low firing rate and short recording time of bat

neurons that could obscure theta rhythmicity (18). To address these issues, our study sought to directly measure intrinsic intracellular mechanisms and showed a lack of theta-frequency properties in layer II neurons with stellate morphology in the bat, unlike the theta-frequency resonance of rat stellate cells. In line with other work (5), this suggests that theta-frequency resonance in the bat cannot provide a mechanism for grid-cell models (27). The lack of continuous theta-rhythmic spiking in the behaving bat and theta-rhythmic intrinsic cellular properties in slices of bat entorhinal cortex suggest that continuous theta may not be a general cross-species physiological mechanism in the mammalian entorhinal cortex and hippocampus (5, 17). Bat neurons do show a peak in the impedance profile below 2 Hz, hinting that grid-cell mechanisms could involve lower frequencies, but the low resonance strength in bat corresponds to smaller rebound depolarization that reduces the capacity to generate grid-cell periodicity in both oscillatory interference and attractor models (28).

Previous data indicate that I_h in rodent stellate cells is critical for theta-frequency sMPR (8, 24). Our recordings from layer II neurons in bat mEC reveal a notable sag response and expression of

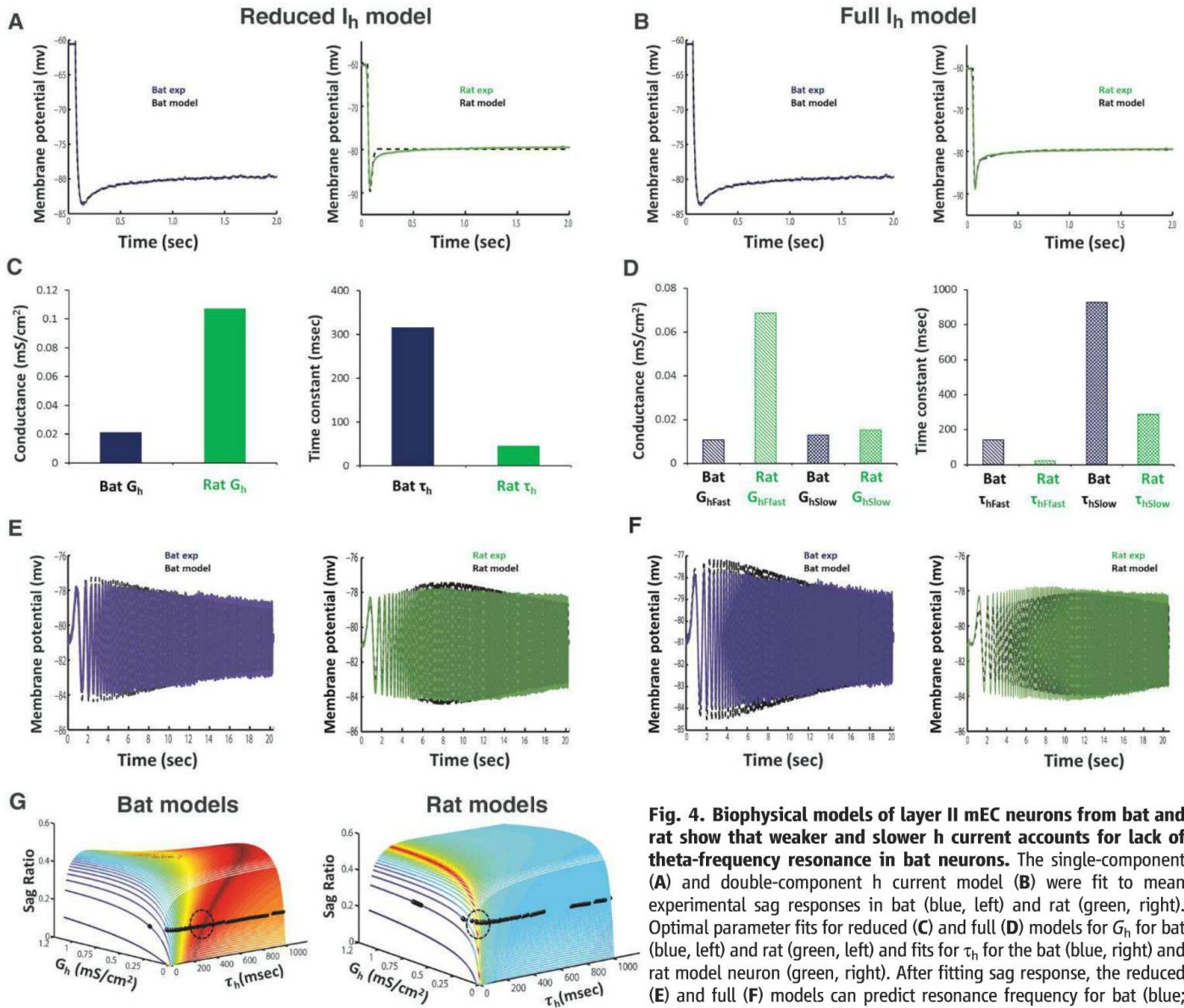


Fig. 4. Biophysical models of layer II mEC neurons from bat and rat show that weaker and slower h current accounts for lack of theta-frequency resonance in bat neurons. The single-component (A) and double-component h current model (B) were fit to mean experimental sag responses in bat (blue, left) and rat (green, right). Optimal parameter fits for reduced (C) and full (D) models for G_h for bat (blue, left) and rat (green, left) and fits for τ_h for the bat (blue, right) and rat model neuron (green, right). After fitting sag response, the reduced (E) and full (F) models can predict resonance frequency for bat (blue; left) and rat (green, right). (G) Sag ratio simulations using different G_h

(1×10^{-6} to 1×10^{-4} S/cm 2) and activation time constants (5 to 1000 ms). Black circles show densities and time constants matching experimental sag ratio for bat (left) and rat (right). Color shows difference between model and experimental sag time constants for bat (left) and rat (right).

I_h . However, our simulations indicate that the non-theta-rhythmic response in the bat is due to smaller and slower h current than in rat stellate cells, similar to the smaller sag seen in nonhuman primates (29). Mice with forebrain-restricted HCN1 knockout (30) exhibit grid cells with larger size and spacing of grid fields compared with those of controls. Knockout of HCN1 reduced theta frequency sMPR (8, 24), suggesting the increase in spacing resulted from decreased resonance frequency and supporting the hypothesis that resonance contributes to grid-cell formation in rodents. However, lack of theta rhythmic resonance in bat suggests an alternative interpretation that theta-frequency sMPR is not necessary for grid cells in the rodent. I_h underlies other neuronal properties, including control of resting membrane potential,

spiking threshold, bistability, normalization of synaptic input, time course of after-hyperpolarization potentials, and synaptic integration and shape of synaptic responses (24, 26, 31–35). I_h could therefore influence grid-cell properties through mechanisms independent of theta-frequency resonance.

Rodentia and Microchiroptera are phylogenetically distant (36), and the absence of theta rhythmicity in bats (5, 17) raises the question of whether grid cells could have arisen independently in the two lineages because of similar selective pressures for spatial navigation. Evidence for convergent neural coding across species can be found in sound localization and olfaction (37–39). Until evidence for convergent evolution in grid-cell formation is established,

slice and whole-animal recordings from bats challenge models of space representation that rely on continuous theta rhythm.

References and Notes

1. M. Fyhn, S. Molden, M. P. Witter, E. I. Moser, M.-B. Moser, *Science* **305**, 1258 (2004).
2. T. Hafting, M. Fyhn, S. Molden, M.-B. Moser, E. I. Moser, *Nature* **436**, 801 (2005).
3. C. F. Doeller, C. Barry, N. Burgess, *Nature* **463**, 657 (2010).
4. N. J. Killian, M. J. Jutras, E. A. Buffalo, *Nature* **491**, 761 (2012).
5. M. M. Yartsev, M. P. Witter, N. Ulanovsky, *Nature* **479**, 103 (2011).
6. C. B. Canto, M. P. Witter, *Hippocampus* **22**, 1277 (2012).
7. L. M. Giocomo, E. A. Zilli, E. Fransén, M. E. Hasselmo, *Science* **315**, 1719 (2007).
8. L. M. Giocomo, M. E. Hasselmo, *J. Neurosci.* **29**, 7625 (2009).

9. A. Boehlen, U. Heinemann, I. Erchova, *J. Neurosci.* **30**, 4585 (2010).
10. J. G. Heys, L. M. Giocomo, M. E. Hasselmo, *J. Neurophysiol.* **104**, 258 (2010).
11. P. D. Dodson, H. Pastoll, M. F. Nolan, *J. Physiol.* **589**, 2993 (2011).
12. T. Hafting, M. Fyhn, T. Bonnevie, M. B. Moser, E. I. Moser, *Nature* **453**, 1248 (2008).
13. A. Jeewajee, C. Barry, J. O'Keefe, N. Burgess, *Hippocampus* **18**, 1175 (2008).
14. H. Stensola *et al.*, *Nature* **492**, 72 (2012).
15. M. P. Brandon *et al.*, *Science* **332**, 595 (2011).
16. J. Koenig, A. N. Linder, J. K. Leutgeb, S. Leutgeb, *Science* **332**, 592 (2011).
17. N. Ulanovsky, C. F. Moss, *Nat. Neurosci.* **10**, 224 (2007).
18. C. Barry, D. Bush, J. O'Keefe, N. Burgess, *Nature* **488**, E1, discussion E2 (2012).
19. E. H. Buhl, J. F. Dann, *Hippocampus* **1**, 131 (1991).
20. C. W. Gattome, L. Slomianka, D. K. Mwangi, H.-P. Lipp, I. Amrein, *Brain Struct. Funct.* **214**, 375 (2010).
21. A. Alonso, R. R. Llinás, *Nature* **342**, 175 (1989).
22. A. Alonso, R. J. Klink, *J. Neurophysiol.* **70**, 128 (1993).
23. C. T. Dickson *et al.*, *J. Neurophysiol.* **83**, 2562 (2000).
24. M. F. Nolan, J. T. Dudman, P. D. Dodson, B. Santoro, *J. Neurosci.* **27**, 12440 (2007).
25. J. S. Haas, A. D. Dorval 2nd, J. A. White, *J. Comput. Neurosci.* **22**, 161 (2007).
26. J. T. Dudman, M. F. Nolan, *PLOS Comput. Biol.* **5**, e1000290 (2009).
27. L. M. Giocomo, M.-B. Moser, E. I. Moser, *Neuron* **71**, 589 (2011).
28. Z. Navratilova, L. M. Giocomo, J. M. Fellous, M. E. Hasselmo, B. L. McNaughton, *Hippocampus* **22**, 772 (2012).
29. C. A. Buckmaster, H. Eichenbaum, D. G. Amaral, W. A. Suzuki, P. R. Rapp, *J. Neurosci.* **24**, 9811 (2004).
30. L. M. Giocomo *et al.*, *Cell* **147**, 1159 (2011).
31. D. L. F. Garden, P. D. Dodson, C. O'Donnell, M. D. White, M. F. Nolan, *Neuron* **60**, 875 (2008).
32. J. C. Magee, *Nat. Neurosci.* **2**, 848 (1999).
33. T. Berger, M. E. Larkum, H. R. Lüscher, *J. Neurophysiol.* **85**, 855 (2001).
34. S. R. Williams, S. R. Christensen, G. J. Stuart, M. Häusser, *J. Physiol.* **539**, 469 (2002).
35. N. A. Otmakhova, J. E. Lisman, *J. Neurophysiol.* **92**, 2027 (2004).
36. W. J. Murphy *et al.*, *Nature* **409**, 614 (2001).
37. L. M. Kay, M. Stopfer, *Semin. Cell Dev. Biol.* **17**, 433 (2006).
38. J. W. Schnupp, C. E. Carr, *Nat. Neurosci.* **12**, 692 (2009).
39. C. Y. Su, K. Menuz, J. R. Carlson, *Cell* **139**, 45 (2009).

Acknowledgments: We thank J. Ahn, J. Barcelo, C. Carr, W. Chapman, and M. Roesch for technical assistance and N. Spruston, J.-W. Lin, H. Eichenbaum, C. Stern, T. Gardner, and N. Ulanovsky for advice and comments. N. Ulanovsky and Y. Yovel helped us acquire Egyptian fruit bats for studies in supplementary materials. Research supported by Office of Naval Research grant N00014-12-1-0339 and Multi-disciplinary University Research Initiative N00014-10-1-0936.

Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6130/363/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S3
References (40–45)

10 December 2012; accepted 27 February 2013
10.1126/science.1233831

Representation of Three-Dimensional Space in the Hippocampus of Flying Bats

Michael M. Yartsev and Nachum Ulanovsky*

Many animals, on air, water, or land, navigate in three-dimensional (3D) environments, yet it remains unclear how brain circuits encode the animal's 3D position. We recorded single neurons in freely flying bats, using a wireless neural-telemetry system, and studied how hippocampal place cells encode 3D volumetric space during flight. Individual place cells were active in confined 3D volumes, and in >90% of the neurons, all three axes were encoded with similar resolution. The 3D place fields from different neurons spanned different locations and collectively represented uniformly the available space in the room. Theta rhythmicity was absent in the firing patterns of 3D place cells. These results suggest that the bat hippocampus represents 3D volumetric space by a uniform and nearly isotropic rate code.

Navigation is crucial for survival, and the need to navigate cuts across the animal kingdom. Several navigational strategies are used by animals, among them maplike navigation (1). This strategy relies on a set of brain structures, at the hub of which is the hippocampus (1, 2). This brain area contains "place cells," neurons that activate when the animal enters a restricted region of the environment, the place field (1, 2). Since the discovery of place cells in rodents, these neurons have been reported across mammalian species (1–9), and their functional properties have been extensively researched (1, 2). However, the spatial and temporal properties of place cells have never been studied in animals moving freely through 3D volumetric space, without any constraints to particular

planes of motion. Indeed, in all studies to date, animals have always been navigating on one- or two-dimensional (2D) planes (1, 10)—either horizontal (5, 7, 11–15), tilted (3, 4, 16–18), or vertical (9, 13, 16)—and thus it remains unresolved how place cells encode the animal's position throughout the entire volume of a 3D volumetric space. We developed wireless recording methodology for freely flying animals (19) and recorded single-neuron activity from hippocampal area CA1 of Egyptian fruit bats flying through 3D space.

Bats were tested in one of two setups: either a large cuboid-shaped flight room, where bats performed a naturalistic foraging task (Fig. 1A and fig. S1; room size 580 × 460 × 270 cm; *n* = 3 bats) or a 3D cubic flight arena of smaller dimensions, where bats searched randomly for food (fig. S2; room size 290 × 280 × 270 cm; *n* = 2 bats) (19). In both setups, bats exhibited complex naturalistic flight trajectories, during which they moved through all the three dimen-

sions of the room (Fig. 1B and fig. S3), traversed large distances (Fig. 1C, left), and flew at high speeds (Fig. 1C, right; and fig. S4). These flight maneuvers resulted in dense and rather uniform coverage of the environment's 3D volume (figs. S5 and S6).

Single-unit activity was recorded from freely flying bats, using a tetrode-based microdrive and a custom lightweight four-channel neural telemetry system designed for flying bats (Fig. 1, D to F, and figs. S7 to S10). The telemetry system allowed the transmission of action potentials from the four channels of one tetrode, with high fidelity (Fig. 1, D, E, and G, and figs. S7 to S9), throughout all the locations in the flight room (figs. S7 and S8) and with very little interference from movement-related noise (fig. S11) (20).

The ability to monitor 3D spatial position and record the activity of individual neurons in freely flying bats allowed studying the spatial coding of 3D volumetric space by hippocampal neurons. We recorded a total of 139 well-isolated neurons from five bats in the dorsal CA1 region of the hippocampus (19). About half of the cells [73 out of 139 (73/139) or 53%] were active during flight, and 75% of the active cells (55/73) were classified as place cells (fig. S12), becoming active when the bat flew through a restricted volume of the available environment (19). Figure 2, A to E, shows the spatial spiking activity of a single hippocampal neuron recorded during flight. This neuron fired nearly exclusively in a confined region of the environment, and this region was restricted in all three dimensions (Fig. 2, A and B, and fig. S13). The firing field of this neuron remained highly stable across the recording session, both in its spatial location and its firing rate (Fig. 2, C and D, and fig. S14A); furthermore, the cell was reliably activated on most of the individual flight passes through the place field (Fig. 2E). Likewise, the majority (75%) of neurons that were active during flight

Department of Neurobiology, Weizmann Institute of Science, Rehovot 76100, Israel.

*Corresponding author. E-mail: nachum.ulanovsky@weizmann.ac.il

exhibited strong spatial tuning (Fig. 2, F to K, and fig. S15), and their firing fields were highly stable throughout the recording session (fig. S14, B and C)—similar to previous reports for rat CA1 place cells (1, 22–24).

In both setups, the place fields of different neurons varied in size and location and spanned much of the available 3D space (Fig. 3, A to D). Most place cells discharged within a single 3D firing field (62%; Fig. 3, E and F). The fraction of cells with two or more fields was higher in the large cuboid flight room (43% or 15/35, Fig. 3E) than in the smaller cubic enclosure (30% or 6/20 cells; Fig. 3F; bootstrap test, $P < 0.001$) (19), which is in agreement with previous reports in rats, where the number of 2D firing fields increased with the size of the environment (21).

Next we asked whether 3D space is represented isotropically in the hippocampus of flying bats; namely, do place fields have the same size in all dimensions? Across the population, the sizes of individual place fields along each of the three cardinal axes of the room were quite similar (Fig. 3, G and H). Utilizing the 3D volumetric coverage of the room by the bats (fig. S1C and fig. S15), we then asked whether 3D place fields are spherical (isotropic), or perhaps anisotropy might be found along some noncardinal axis. We fitted a 3D ellipsoid to the place-field shape, where the ellipsoid was not constrained to be parallel to any of the room walls, and an “elongation index” was defined as the ratio of the longest to the shortest axes of the ellipsoid (fig. S16). To test for the significance of the computed elongation index, we implemented a shuf-

fling procedure in which we randomly redistributed the spikes of the place field along the bat's flight trajectory, constraining them to be within a perfect sphere of the same volume as the ellipsoid, and tested whether the real elongation index was outside the shuffling-based confidence interval (19); that is, whether the actual elongation was significantly different from that expected from an underlying perfect sphere, given the animal's behavior (Fig. 3I). Only 9% of the fields (Fig. 3I, red dots; 7/82 fields) showed significant elongation, exhibiting compression along the z axis of the room (e.g., neuron 1 in fig. S17C), but sometimes also diagonal field shapes (e.g., neuron 2 in fig. S17C). However, 91% of the place fields in both arenas (75/82 fields) were not significantly different from an underlying sphere (Fig. 3I, green dots; examples in fig. S17C, neurons 3

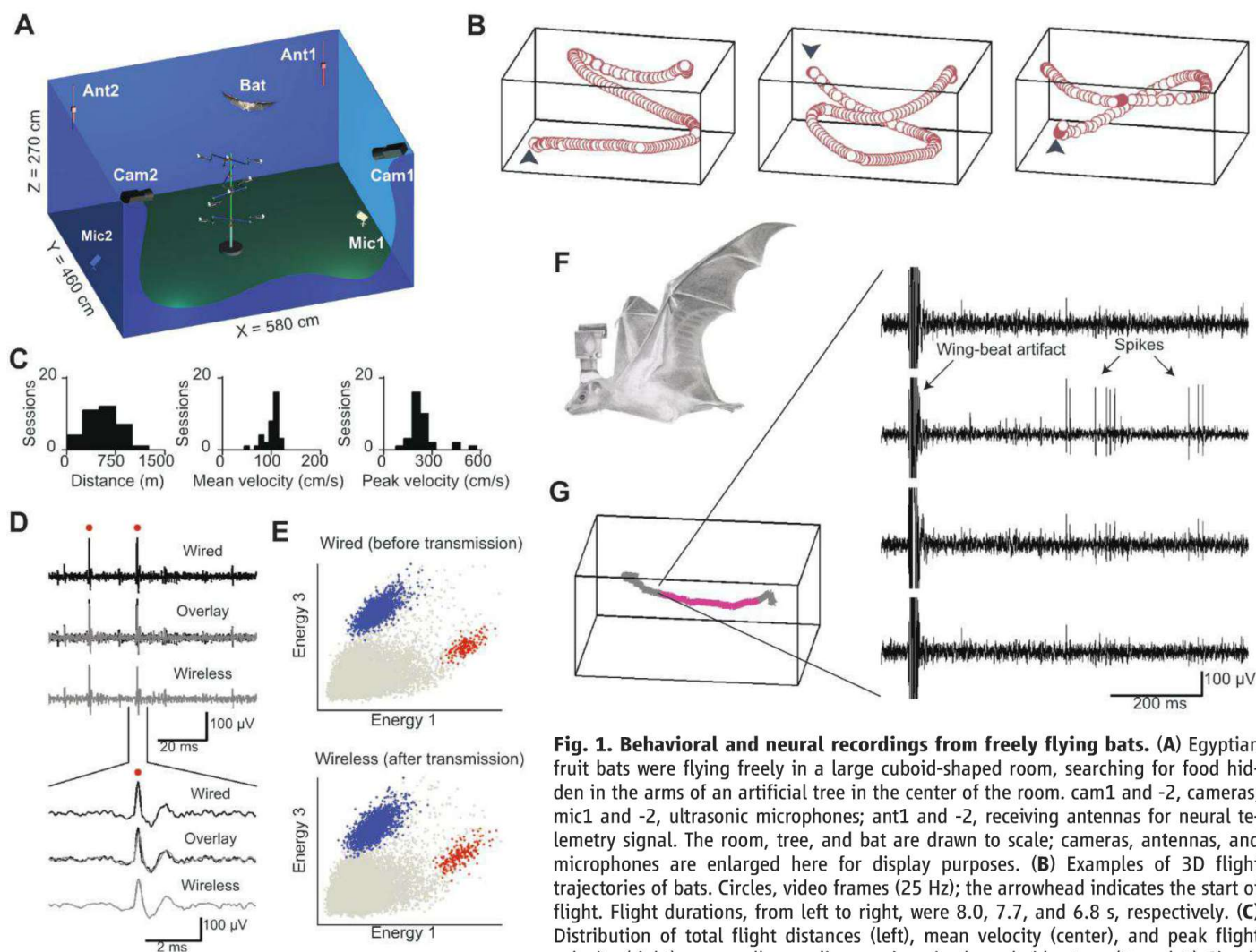


Fig. 1. Behavioral and neural recordings from freely flying bats. (A) Egyptian fruit bats were flying freely in a large cuboid-shaped room, searching for food hidden in the arms of an artificial tree in the center of the room. cam1 and -2, cameras; mic1 and -2, ultrasonic microphones; ant1 and -2, receiving antennas for neural telemetry signal. The room, tree, and bat are drawn to scale; cameras, antennas, and microphones are enlarged here for display purposes. (B) Examples of 3D flight trajectories of bats. Circles, video frames (25 Hz); the arrowhead indicates the start of flight. Flight durations, from left to right, were 8.0, 7.7, and 6.8 s, respectively. (C) Distribution of total flight distances (left), mean velocity (center), and peak flight velocity (right) across all recording sessions in the cuboid setup. (D and E) Simultaneous wired (tethered) and wireless neural recordings. (D) Top: Raw neural trace recorded from bat hippocampal area CA1 from a single channel of the tetrode, before transmission (top), after transmission (bottom), and overlay (center). Bottom: Zoom-in onto a single action potential. (E) 2D projections of spike energies of CA1 neurons, before (top) and after (bottom) wireless transmission. Red and blue dots, clusters corresponding to two neurons; red dots in (E) are spikes from the red cluster. (F) Telemetry system on a flying bat, drawn to scale [illustration: S. Kaufman]. (G) Neural traces (right) from the four channels of a tetrode, recorded from bat CA1 during one flight segment (left, gray; the magenta portion corresponds to the duration of the neural traces shown on the right).

and 4). Further, comparing the field sizes specifically along the x, y, z cardinal axes of the room showed that, for these 91% of the fields, there was no significant elongation in any of the cardinal directions (fig. S17A; Sign test, $P > 0.15$ for all comparisons). Thus, for the large majority of neurons, 3D place fields were indistinguishable from isotropic spheres.

We next examined whether the locations and sizes of place fields are distributed uniformly across space, as indicated by the population plots in Fig. 3, A to D. Indeed, the size of place fields did not show significant correlation with the distance from the flight-enclosure center, in either of the setups, indicating similar resolution at room center and near the walls (Fig.

3J; correlation coefficient: $r = -0.20$, $P = 0.13$ for the rectangular cuboid flight-room; $r = -0.02$, $P = 0.91$ for the cubic enclosure). Place fields were slightly bigger in the large cuboid flight room than in the smaller cubic flight enclosure (102.3 ± 5.5 cm compared to 94.3 ± 6.9 cm, respectively). Field locations were distributed quite uniformly, both along the radial

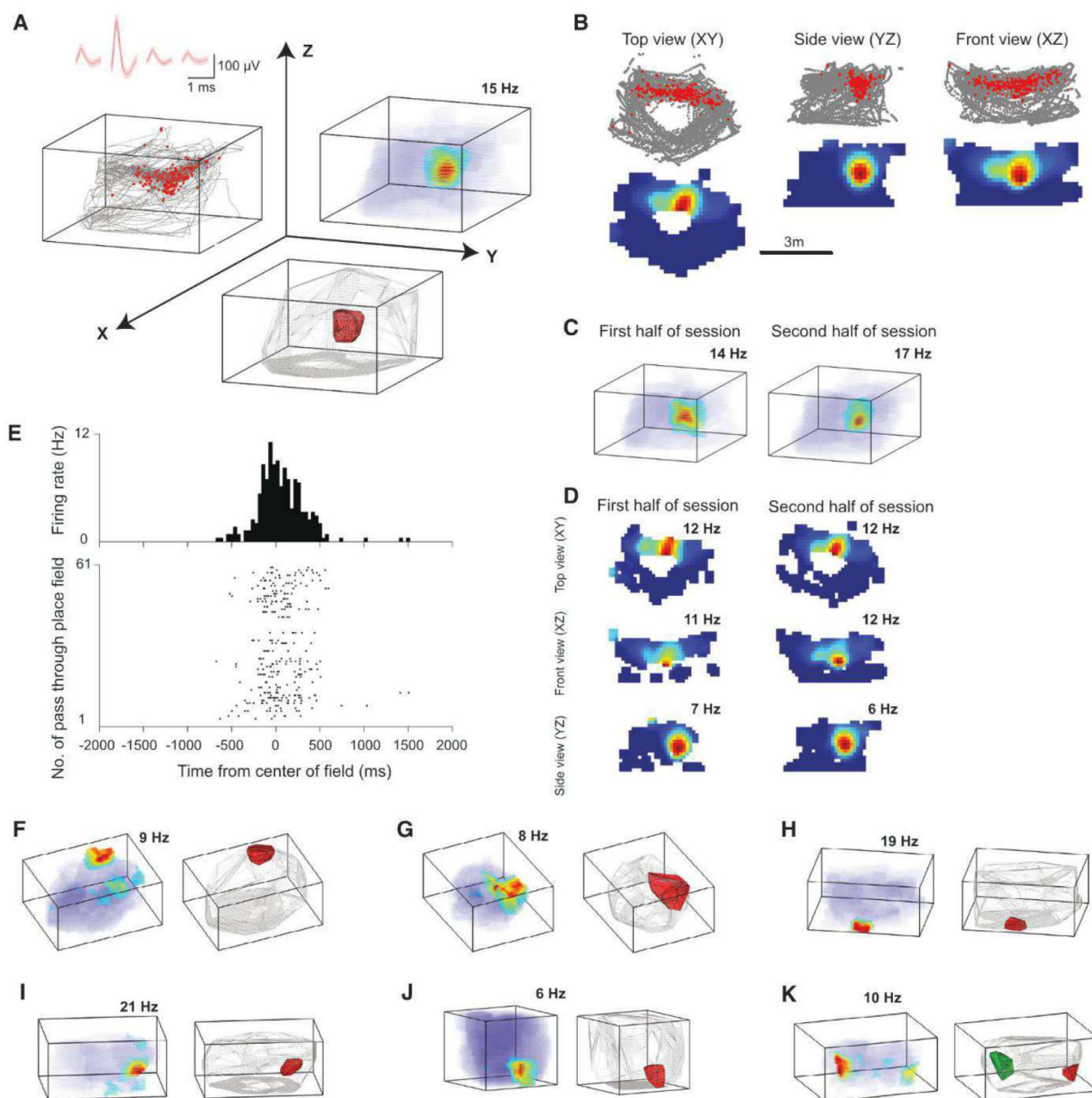


Fig. 2. Examples of 3D place cells recorded from the hippocampus of flying bats. (A to E) An example cell. (A) 3D representation of the neuron's spatial firing. Top left: Spikes (red dots) overlaid on bat's position (gray lines); shown also are the spike waveforms on the four channels of the tetrode (mean $\pm$ SD). Top right: 3D color-coded rate map, with peak firing rate indicated. Bottom: Convex hull encompassing the neuron's place field (red polygon) and the volume covered by the bat during flight (gray polygon). (B) 2D projections of the raw data (top) and color-coded rate maps (bottom). (C and D) Stability of the neuron's spatial firing for the first versus second half

of the recording session; shown are 3D rate maps (C) and 2D projections (D). Peak firing rate is indicated for each map. (E) Reliability of firing across 61 flight passes through the place field. Bottom: raster plot showing spikes during individual passes (time 0, point closest to field center). Top: Spike density function, unsmoothed; bin size, 40 ms. (F to K) Six additional place cells from the hippocampus of different bats. Same notation as in (A). When a neuron had more than one place field, different fields were marked with different colors (K). The neuron in (J) was recorded in the cubic enclosure; the other neurons are from the rectangular-cuboid room.

dimension (Fig. 3J) and along each of the three major axes (Fig. 3, K and L), indicating that the entire available environment was represented rather uniformly by the population of place cells.

Most hippocampal neurons in rodents exhibit strong theta modulation of their firing rate, which can be readily observed in spike-train

temporal autocorrelograms (22–25). However, it has been difficult to find firing-rate oscillations in the hippocampus of several other mammals, including wheeling monkeys (26) and crawling bats [(3, 5); fig. S18]. But could theta rhythmicity occur during flight? We hypothesized this might be the case, because flight entails several

conditions favorable for the generation and detection of theta rhythmicity, such as high-velocity movements (fig. S4), high firing rates (Fig. 2 and fig. S19), and strong oscillatory behaviors by the animal (fig. S20). Surprisingly, however, we found the opposite. The theta rhythmicity of hippocampal place cells was assessed by computing

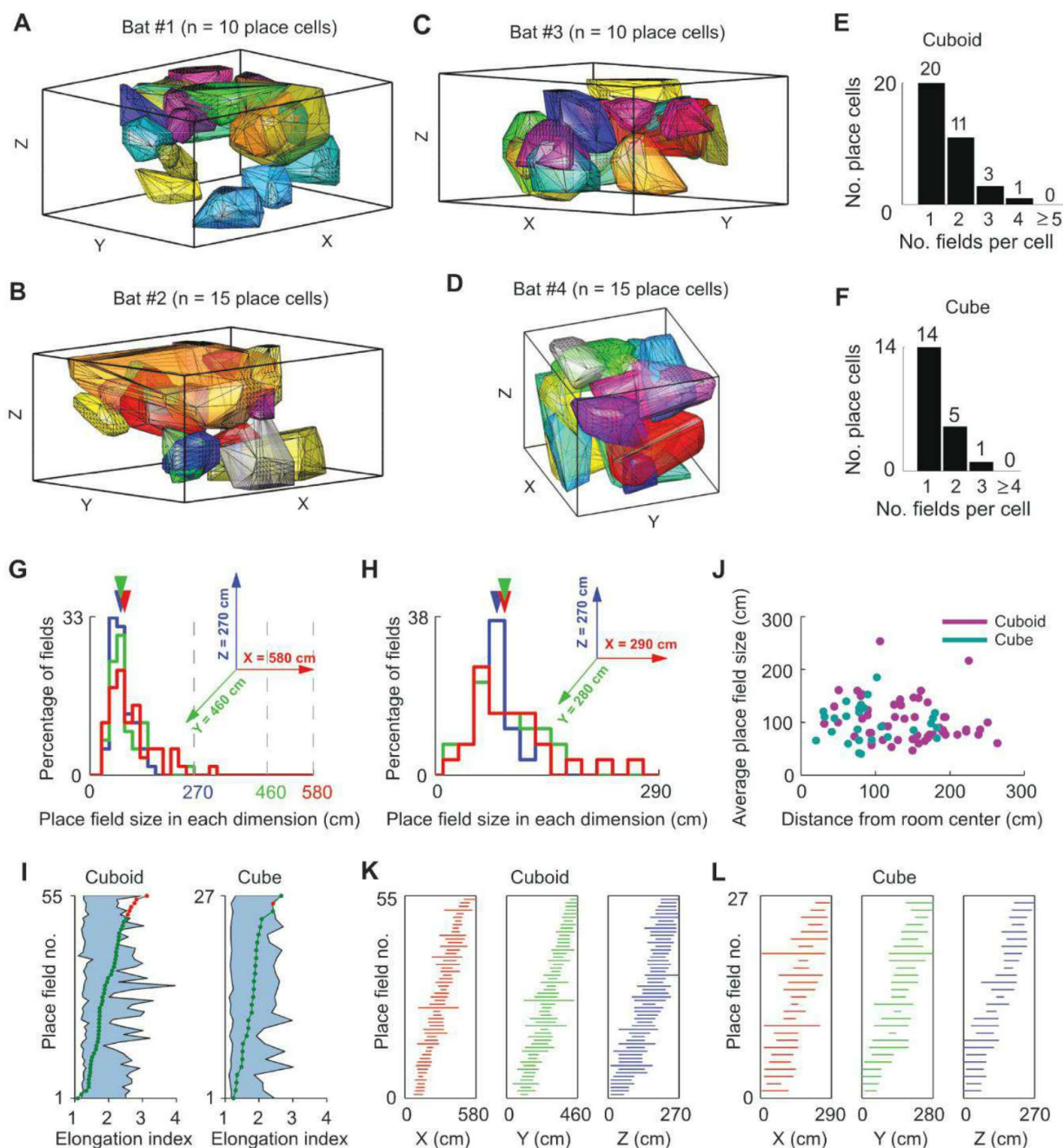


Fig. 3. 3D space is encoded uniformly and nearly isotropically in the hippocampus of flying bats. (A to D) All the place fields recorded from the hippocampus of four individual bats (different colors denote different neurons). Bats 1 to 3 (A) to (C) were tested in the cuboid-shaped flight-room, bat 4 (D) in the cubic enclosure. (E and F) Distribution of the number of place fields per neuron, in each experimental setup. (G and H) Distribution of place-field size in each dimension (different colors), in the rectangular-cuboid (G) and cubic setup (H). Color-matched arrowheads indicate median values. (I) Elongation indices for all place fields in both setups, sorted in

ascending order. Shaded area, 95% confidence intervals (19). Only a small fraction of place fields had a shape significantly different from a sphere (9%, or 7/82 fields, red dots), whereas 91% of place fields were not statistically different from a sphere (green dots). (J) Average place-field size versus distance from room center in both setups (different colors); each symbol represents a single place field. (K and L) Locations and sizes of all place fields in each dimension of the room (x,y,z) in the cuboid (K) and cubic (L) setup; each horizontal line represents the extent of a single place field, sorted by position separately for x,y,z; same colors as in (G) and (H).

a standard “theta index” (Fig. 4, A to C, and fig. S21) (19), which has been used in previous studies in rats and bats to assess theta rhythmicity (5, 23, 24). A statistical shuffling procedure was applied for each individual neuron to test for significance (or lack thereof) of theta modulation in bat hippocampal neurons (19). Only 3.6% of the 3D place cells in both experimental setups (2/55 neurons) exhibited significant theta-modulated firing (19), but this rhythmicity was very weak (fig. S21) and, at the population level, was not different than would be expected by chance (binomial test, $P = 0.77$) (19). The degree of theta modulation of bat hippocampal neurons was stably low when comparing crawling versus the two flight setups (Fig. 4D), despite dramatic increases in movement velocity (speeding up almost two orders of magnitude: Fig. 4, D and E, and figs. S4 and S19) and a much higher firing rate of neurons during rapid flight (Fig. 4E and fig. S19). Theta rhythmicity was not correlated with the bat’s echolocation rate (fig. S21H). Thus, we found a near-absence of spike-train theta rhythmicity in the bat hippocampus, both

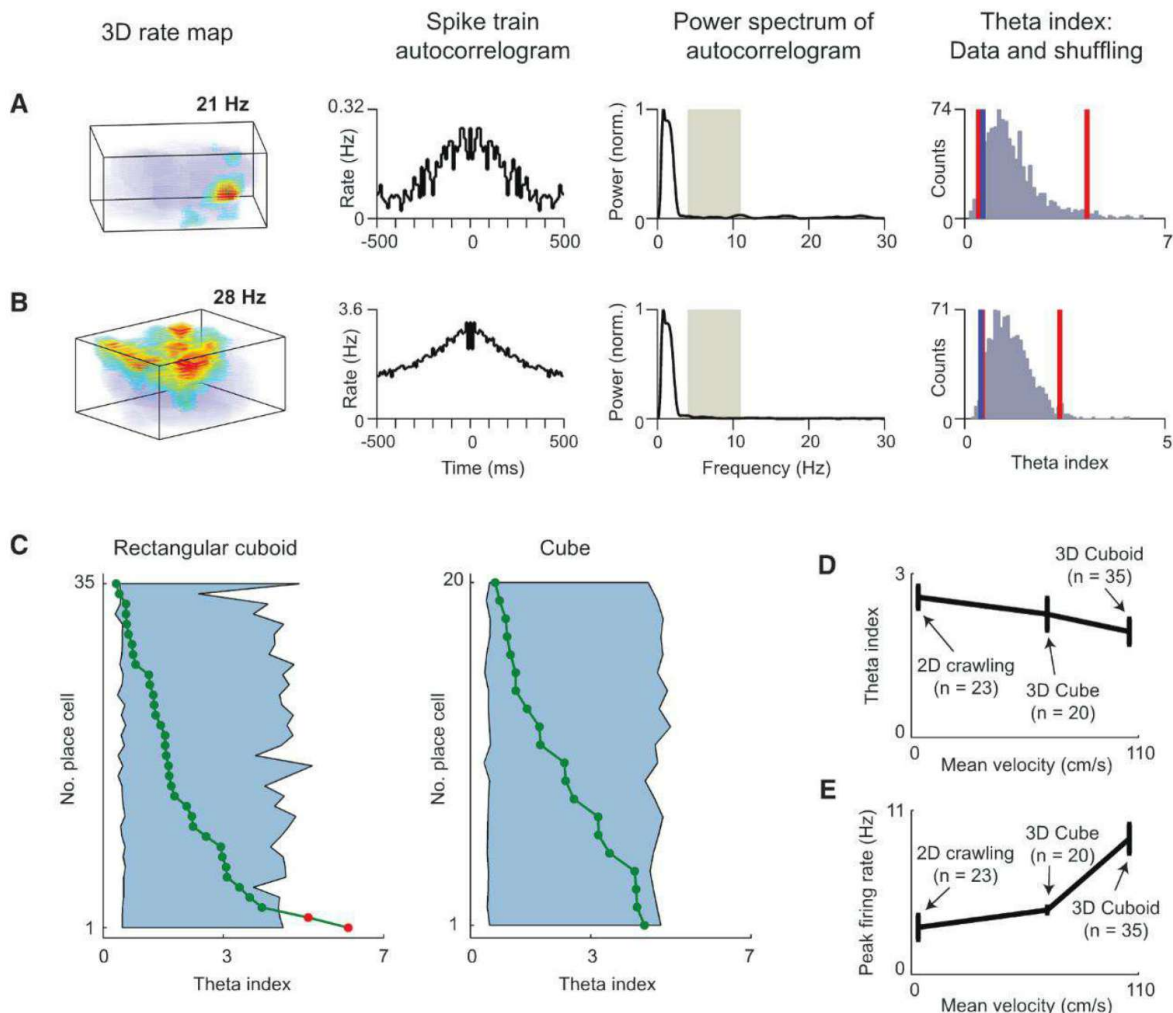
during crawling and during free flight. These results are strikingly different from the robust theta-rhythmic spiking of place cells in rodents (1) but are consistent with our previous reports in crawling bats (3, 5). The near absence of theta modulation in the firing of hippocampal place cells in bats (this study) and monkeys (26) is incongruent with dual-oscillatory models of spatial coding (1, 2).

What factors may shape the nearly isotropic 3D coding that we found in >90% of bat hippocampal place cells during flight? Bats are mammals that naturally move and navigate in 3D volumetric environments, and additionally their echolocation system provides them with volumetric 3D sensory information, including depth (10, 27). Thus, isotropic 3D spatial coding might have evolved in bats because of the pressures to encode and retrieve 3D sensory information. This interpretation also implies possibly different encoding of 3D space in land-dwelling mammals that typically do not move volumetrically in 3D during ontogeny but instead rely mainly on horizontal self-motion cues. Indeed,

a recent study (16) reported anisotropic coding in rat hippocampal neurons, with strong elongation of place fields in the vertical dimension [but see isotropic coding reported in a different study in rats (9) and discussion in (10, 28)]. However, movement through 3D volumetric spaces is characteristic for many nonflying mammals, and we hypothesize that, as in bats, this could have created an evolutionary pressure to represent 3D volumetric position in the brain circuits of many species. The extent to which this prediction applies to humans is an intriguing question, because on one hand we evolved from monkeys that jumped across 3D volumes, but on the other hand we typically locomote along 2D surfaces. This will have to be studied in humans and monkeys in real and virtual 3D settings.

Finally, would these findings extend also to real-life navigation in the wild (29)? The average size of 3D place fields found here (~1 m) was consistent with the predictions of a computation model of 3D place cells, for room-sized environments (30); the same model also predicts place fields on the order of kilometers

Fig. 4. Near-absence of theta rhythmicity in the firing of bat hippocampal place cells during flight. (A and B) Two example cells. From left to right: 3D color-coded rate maps, with peak firing rate indicated; spike-train temporal autocorrelograms; power spectrum of autocorrelogram; and shuffled distribution of theta indexes (red lines, 95% confidence intervals; blue line, actual theta index of the neuron) (19). There was a near-absence of power in the theta band (5 to 11 Hz, gray rectangle in the third column; power normalized to 1). See fig. S21 for additional examples. (C) Theta indexes for all place cells from the rectangular-cuboid flight room (left) and cubic flight enclosure (right). Neurons are sorted by theta-index values. Shaded area, 95% confidence intervals. Only a small fraction of place cells were significantly theta-modulated (red dots: 3.6%, or 2/55 of neurons in both setups together), whereas nearly all the place cells were not modulated (green dots: 96.4%, or 53/55 of neurons). (D and E) Theta indexes (D) and peak firing rates (E) as a function of the bat’s velocity, under three different conditions (crawling, flying inside the cubic enclosure, or flying in the cuboid flight room).



in bats flying in their natural habitat. Further, navigation requires more than the hippocampal spatial signal: It also entails decision-making, goal-directed behaviors, sensory-motor integration, and other cognitive processes (mediated by brain structures such as the striatum, cerebellum, and prefrontal and parietal cortices). Thus, to elucidate the neural basis of real-life navigation in bats (27, 29), it would be essential to record neural activity from the hippocampal formation (and additional brain structures) in bats navigating over distances of kilometers.

References and Notes

1. P. Andersen, R. G. Morris, D. Amaral, T. Bliss, J. O'Keefe, *The Hippocampus Book* (Oxford Univ. Press, New York, 2007).
2. E. I. Moser, E. Kropff, M.-B. Moser, *Annu. Rev. Neurosci.* **31**, 69 (2008).
3. N. Ulanovsky, C. F. Moss, *Nat. Neurosci.* **10**, 224 (2007).
4. N. Ulanovsky, C. F. Moss, *Hippocampus* **21**, 150 (2011).
5. M. M. Yartsev, M. P. Witter, N. Ulanovsky, *Nature* **479**, 103 (2011).
6. T. Ono, K. Nakamura, H. Nishijo, S. Eifuku, *J. Neurophysiol.* **70**, 1516 (1993).
7. C. G. Kentros, N. T. Agnihotri, S. Streater, R. D. Hawkins, E. R. Kandel, *Neuron* **42**, 283 (2004).
8. A. D. Ekstrom et al., *Nature* **425**, 184 (2003).
9. J. J. Knierim, B. L. McNaughton, G. R. Poe, *Nat. Neurosci.* **3**, 209 (2000).
10. N. Ulanovsky, *Curr. Biol.* **21**, R886 (2011).
11. C. D. Harvey, F. Collman, D. A. Dombeck, D. W. Tank, *Nature* **461**, 941 (2009).
12. E. J. Henriksen et al., *Neuron* **68**, 127 (2010).
13. N. Ludvig, H. M. Tang, B. C. Gohil, J. M. Botero, *Brain Res.* **1014**, 97 (2004).
14. M. A. Wilson, B. L. McNaughton, *Science* **261**, 1055 (1993).
15. S. P. Jadhav, C. Kemere, P. W. German, L. M. Frank, *Science* **336**, 1454 (2012).
16. R. Hayman, M. A. Verriotes, A. Jovalekic, A. A. Fenton, K. J. Jeffery, *Nat. Neurosci.* **14**, 1182 (2011).
17. J. J. Knierim, B. L. McNaughton, *J. Neurophysiol.* **85**, 105 (2001).
18. K. J. Jeffery, R. L. Anand, M. I. Anderson, *Exp. Brain Res.* **169**, 218 (2006).
19. Materials and methods are available as supplementary materials on Science Online.
20. This telemetry system did not allow recording of low-frequency local field potentials because of inherent high-pass limitations (19). The bat's wings hitting on the telemetry transmitter caused some recording artifacts (Fig. 1G and fig. S11), but these artifacts constituted only a small fraction of the flight time ($3.6 \pm 0.91\%$ of the total flight time per day, mean $\pm$ SD). This fraction decayed to 0.11% after 9 recording days (fig. S11D), enabling us to collect neural spiking data with almost no movement-related interference.
21. A. A. Fenton et al., *J. Neurosci.* **28**, 11250 (2008).
22. S. Royer, A. Sirota, J. Patel, G. Buzsáki, *J. Neurosci.* **30**, 1777 (2010).
23. R. F. Langston et al., *Science* **328**, 1576 (2010).
24. T. J. Wills, F. Cacucci, N. Burgess, J. O'Keefe, *Science* **328**, 1573 (2010).
25. J. Koenig, A. N. Linder, J. K. Leutgeb, S. Leutgeb, *Science* **332**, 592 (2011).
26. E. Hori, E. Tabuchi, N. Matsumura, T. Ono, H. Nishijo, *Front. Behav. Neurosci.* **5**, 36 (2011).
27. N. Ulanovsky, C. F. Moss, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 8491 (2008).
28. J. S. Taube, M. Shinder, *Hippocampus* **23**, 14 (2013).
29. A. Tsour et al., *Proc. Natl. Acad. Sci. U.S.A.* **108**, E718 (2011).
30. W. M. Brown, A. Bäcker, *Neural Comput.* **18**, 1511 (2006).

Acknowledgments: We thank A. Treves, D. Derdikman, J.-M. Fellous, L. Las, Y. Yovel, M. Ahrens, and C. Brody for comments on the manuscript; R. Raz, K. Kamenitz, and M. Geva-Sagiv for the 3D trajectory reconstruction system; C. Stengel for the neural telemetry system; B. Pasmantir and G. Ankaoua for mechanical designs; A. Rubin and A. Averkin for assistance with neural recordings and video calibrations; S. Kaufman, T. Dayan, and S. Dror for bat training; M. Weinberg and A. Tuval for veterinary oversight; C. Ra'anani and R. Eilam for histology; and M. P. Witter for advice on reconstruction of tetrode-track locations. This study was supported by research grants from the European Research Council (ERC-NEUROBAT), the Human Frontiers Science Program (HFSP RGP0062/2009-C), the Israel Science Foundation (ISF 1017/08), and the Minerva Foundation to N.U. and a Lev-Zion predoctoral excellence fellowship to M.M.Y.

Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6130/367/DC1
Materials and Methods
Figs. S1 to S22
References

18 January 2013; accepted 21 February 2013
10.1126/science.1235338

Developmental Decline in Neuronal Regeneration by the Progressive Change of Two Intrinsic Timers

Yan Zou,^{1*} Hui Chiu,^{1*} Anna Zinovyeva,² Victor Ambros,² Chiou-Fen Chuang,^{1†} Chieh Chang^{1,3†}

Like mammalian neurons, *Caenorhabditis elegans* neurons lose axon regeneration ability as they age, but it is not known why. Here, we report that *let-7* contributes to a developmental decline in anterior ventral microtubule (AVM) axon regeneration. In older AVM axons, *let-7* inhibits regeneration by down-regulating LIN-41, an important AVM axon regeneration-promoting factor. Whereas *let-7* inhibits *lin-41* expression in older neurons through the *lin-41* 3' untranslated region, *lin-41* inhibits *let-7* expression in younger neurons through Argonaute ALG-1. This reciprocal inhibition ensures that axon regeneration is inhibited only in older neurons. These findings show that a *let-7*-*lin-41* regulatory circuit, which was previously shown to control timing of events in mitotic stem cell lineages, is reutilized in postmitotic neurons to control postdifferentiation events.

We use *Caenorhabditis elegans* to study developmental decline in neuronal regeneration (Fig. 1A) (1). As in verte-

brates, advancing development leads to decreased axon regenerative capacity in *C. elegans* (Fig. 1C) (2–4). The timing mechanism that controls developmental decline in neuronal regeneration is poorly understood (2–5). Because heterochronic genes are implicated in regulating developmental timing and aging in *C. elegans* (6), we hypothesized that they might regulate developmental decline in neuronal regeneration. The heterochronic pathway involves a number of microRNA-regulated posttranscriptional genetic circuits (7, 8), including an important interaction between the

let-7 microRNA and its direct target, *lin-41*, which encodes a tripartite motif (TRIM) protein (9, 10). We show here that *let-7* and *lin-41* function in postmitotic neurons to time their differentiation and postdifferentiation events. Our study reveals that the intrinsic timing mechanism that controls developmental decline in neuronal regeneration depends on the progressive increase of *let-7* and the progressive decrease of *lin-41* in neurons, with *let-7*-*lin-41* reciprocal inhibition having a role in this process.

MicroRNA expression is either spatially restricted or temporally regulated in neuronal development (11–14). To explore the role of microRNAs in neuronal regeneration, we examined anterior ventral microtubule (AVM) axon regeneration in mutants defective in microRNA biogenesis, *dcr-1* and *alg-1* (15). Although *dcr-1* hypomorphic mutant animals displayed normal AVM axon regeneration (fig. S1A), regenerating AVM neurons of adult *alg-1* mutants extended axons 2.5 times the length of those in adult wild-type animals (Fig. 1, B to D). In addition, regenerating axons of *alg-1* adults often displayed compact growth cones (fig. S1C). These regeneration phenotypes in adult *alg-1* mutants are reminiscent of those in wild-type animals at an earlier developmental stage (Fig. 1, B to D, and fig. S1), which suggests that *alg-1* mutations may retard a normal developmental decline in axon regeneration. Consistent with this conclusion, we observe that, although AVM axon regeneration in *alg-1* mutants is similar to that of wild-type animals at the larval L2

¹Division of Developmental Biology, Cincinnati Children's Hospital Research Foundation, Cincinnati, OH 45229, USA.

²RNA Therapeutics Institute and Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01605, USA. ³Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec H3A 1B1, Canada.

*These authors contributed equally to this work.

†Corresponding author. E-mail: chieh.chang@cchmc.org (C.C.); chiou-fen.chuang@cchmc.org (C.-F.C.)

stage (Fig. 1, C and D), during advanced L3-YA (young adult) stages, AVM axon regeneration is significantly reduced in wild-type animals, but is unchanged in *alg-1* mutants until day 4 of the adult stage (Fig. 1, C and D).

We hypothesize that ALG-1-dependent microRNAs are likely to be expressed late to contribute to the observed developmental decline in axon regeneration. Stem-loop reverse transcription polymerase chain reaction (RT-PCR) was used to globally survey mature microRNA expression in *C. elegans* (fig. S2A). Among 90 microRNAs surveyed (fig. S2), we identified 10 late-onset microRNAs whose expression coincides with the developmental decline in axon regeneration (Fig. 2A). Among the 10 late-onset microRNAs identified, only *let-7*, *mir-84*, and *mir-241* are expressed in AVM neurons (Fig. 2, B to H, and fig. S3A). Out of those, only *let-7* maturation is affected in *alg-1* mutants (Fig. 2I and fig. S3B). In our assay using RNA preparations from staged animals, expression of the mature *let-7* microRNA is almost undetectable from embryonic to L2 stages and becomes apparent at L3 (Fig. 2A). To study specifically the expression of *let-7* in AVM, we developed a 2.9-kilobase *let-7* promoter reporter that stably expresses in AVM and whose expression levels in the whole animal at different stages correlate very well with the whole-animal stem-loop RT-PCR results (Fig. 2A and fig. S3C). This *let-7* reporter is expressed at relatively low levels in AVM at L1 and L2 stages (Fig. 2, C and J) but is significantly elevated from L3 onward (Fig. 2, F and J). This indicates that *let-7* is expressed at the right place and right time to contribute to the developmental decline in AVM axon regeneration. Several lines of evidence indicate that the effect of *alg-1* mutations

on AVM axon regeneration can be attributed to *let-7* inactivation in AVM. First, *let-7* mutants display enhanced AVM axon regeneration to a similar extent to *alg-1* mutants (Fig. 2, K to M). Second, the effect of *alg-1* mutations on AVM axon regeneration is rescued by the *let-7* overexpression in AVM (Fig. 2M). Last, although the *alg-1* RNA interference (RNAi) causes enhanced AVM axon regeneration, it does not further enhance regeneration in *let-7* mutants (Fig. 2M).

The temperature-sensitive allele used in this study, *let-7(n2853ts)*, is a point mutation in the 5' seed region of the *let-7* microRNA. *let-7(n2853ts)* animals have normal *let-7* activity at the permissive temperature of 15°C but have reduced *let-7* activity at the nonpermissive temperature of 20°C. In *let-7(n2853ts)* mutants at 20°C, AVM axons regenerated to twice the length of those regenerated in wild-type animals (Fig. 2M), which suggested that *let-7* mutations recapitulate the *alg-1* mutant phenotype in enhancing AVM axon regeneration (Fig. 2M). *let-7(n2853ts)* likely represents a loss-of-function mutation in AVM at 20°C, because *let-7(n2853ts)* mutants display a similar degree of AVM axon regeneration at 23°C as at 20°C (average axon regeneration is 105 μ m at 23°C and 100 μ m at 20°C, $P = 0.32$). Like *alg-1* mutants, *let-7(n2853ts)* animals also display a retarded developmental decline in AVM axon regeneration (fig. S4, A and B). Regenerating AVM neurons in *let-7(n2853ts)* mutants not only extended longer axons, they also frequently displayed compact growth cones (fig. S1D), a phenotype shared by *alg-1* mutants (fig. S1C). Some regenerating axons in *let-7* mutants only stop outgrowth as they are about to enter the nerve ring (Fig. 2L). The effect of *let-7* mutations on AVM axon regeneration appears to be cell

autonomous, as expression of *let-7* in AVM significantly rescued the *let-7* mutant phenotype of enhancing AVM axon regeneration (Fig. 2M).

lin-4, like *let-7*, is a developmental timing microRNA and is expressed strongly in AVM (14). AVM axon regeneration in *lin-4* mutants is not significantly different from that in wild-type animals (fig. S5A), which suggests that different developmental-timing microRNAs play different roles in AVM: *lin-4* is used to time AVM axon connectivity as reported previously (14); *let-7* is used to time developmental decline in AVM axon regeneration.

To determine whether the effect of *let-7* on axon regeneration represents a prior role for *let-7* during development of the AVM to establish its regenerative potential or, rather, an acute requirement for *let-7* during the process of regeneration, we performed temperature-shift experiments. We observed that *let-7(n2853ts)* animals shifted to the nonpermissive temperature after injury exhibit better axon regeneration than wild-type animals (fig. S5). These data indicate that *let-7* has an immediate effect in regenerating AVM neurons to inhibit their regenerative capacity.

let-7 is expressed only weakly in AVM and anterior lateral microtubule (ALM) mechanosensory neurons at the L1 stage (Fig. 2J) when AVM and ALM axon development is under way, which suggests that a low level of *let-7* may be necessary for normal axon development. To test whether elevated *let-7* would inhibit AVM and ALM axon outgrowth in development, we forced early expression of *let-7* in AVM and ALM using a cell-specific *mec-4* promoter, which enables gene expression from the late embryonic stage onward. Forced early expression of *let-7* in AVM and ALM limits their axon outgrowth during

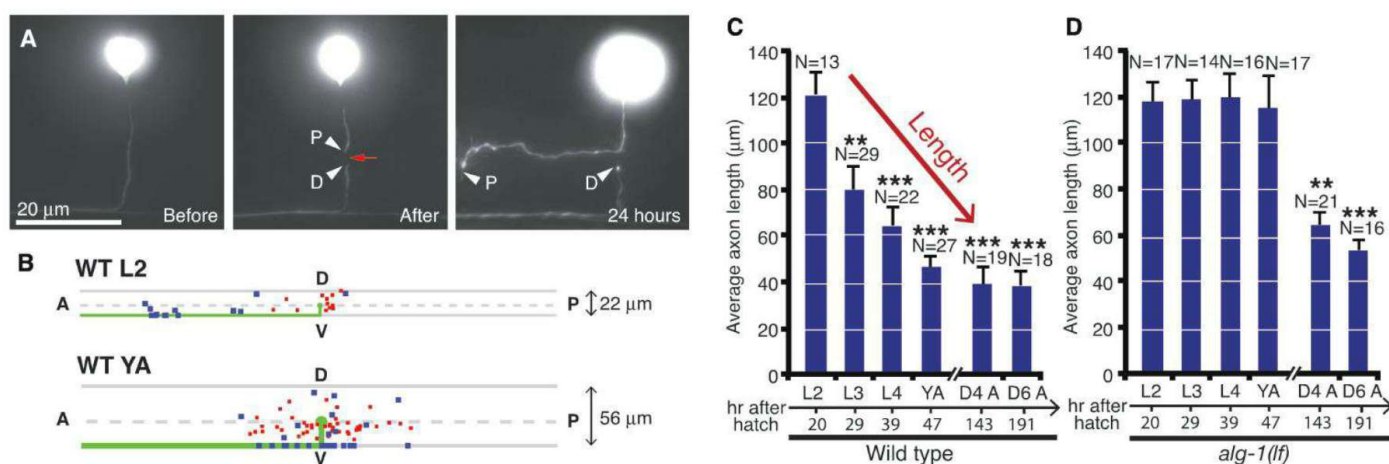


Fig. 1. *alg-1* mutations slow developmental decline in AVM axon regeneration. (A) AVM axon before, immediately after, and 24 hours after laser surgery in a wild-type animal. D (distal end) and P (proximal end) of severed axons. Red arrow points to the focus of laser plasma. Anterior is left, dorsal up. (B) The positions of regenerating axon termini in wild-type L2-stage animals, wild-type YAs, and *alg-1* YAs. In each scatter plot, the top line (dorsal nerve cord), the bottom line (ventral nerve cord), and the wild-type AVM morphology before surgery (green) are shown. (C and D) Average AVM axon length in wild type (C) and *alg-1* mutants (D) at different stages. Asterisks indicate cases in which latter stage animals differ from L2-stage animals at $**P < 0.01$; $***P < 0.001$. Error bars indicate SEM.

animals, wild-type YAs, and *alg-1* YAs. In each scatter plot, the top line (dorsal nerve cord), the bottom line (ventral nerve cord), and the wild-type AVM morphology before surgery (green) are shown. (C and D) Average AVM axon length in wild type (C) and *alg-1* mutants (D) at different stages. Asterisks indicate cases in which latter stage animals differ from L2-stage animals at $**P < 0.01$; $***P < 0.001$. Error bars indicate SEM.

development (fig. S6, A to G), which suggests that premature *let-7* expression closes early the window of plasticity for axon outgrowth.

hbl-1 and *lin-41* are critical direct targets of *let-7* in *C. elegans* developmental timing (10, 16). *hbl-1* encodes a transcription factor and LIN-41 is a member of TRIM family of proteins. Analysis of the promoter activity of *hbl-1* and *lin-41* genes by using green fluorescent protein–fused promoter (promoter::GFP) reporters showed that *lin-41* is expressed in AVM and ALM, but *hbl-1* is not (Fig. 3A). Also, *lin-41* mutants (but not *hbl-1* mutants) display significantly reduced AVM axon regeneration (Fig. 3, C and E). *lin-41* mutations suppress the *let-7* mutant phenotype of enhancing AVM axon regeneration, which suggests that *lin-41* acts downstream of *let-7* in AVM (Fig. 3E). Consistent with a positive role for *lin-41* in promoting axon regeneration, overexpressing *lin-41* in AVM enhanced AVM axon regeneration (Fig. 3, D and E).

Consistent with a developmental down-regulation of *lin-41* in AVM by rising *let-7*, an engineered genomic reporter containing the 5', coding, and 3' fragments of the *lin-41* gene showed a stage-dependent decline in AVM (Fig. 3B). This down-regulation of *lin-41* is likely via

direct interaction of *let-7* with the *lin-41* 3' untranslated region (3'UTR), as the expression of a *GFP::lin-41* 3'UTR sensor in adult AVM was higher in *let-7* mutants than in wild-type animals (fig. S7), and ectopic expression of *let-7* in younger AVM was sufficient to repress *GFP::lin-41* 3'UTR (fig. S8).

lin-29, a zinc finger transcription factor of the C2H2 type (17), is known to be inhibited by *lin-41* (10). We show that *lin-29* mutations cause enhanced AVM axon regeneration but do not further enhance regeneration in *let-7* mutants (Fig. 3E). In addition, *lin-29* mutations suppress the *lin-41* mutant phenotype of reducing AVM axon regeneration (Fig. 3E). Further, the level of the LIN-29::GFP fusion protein in AVM at the L1 stage is significantly higher in *lin-41* mutants than in wild-type animals (Fig. 3F). Together, our results indicate that *let-7*/*lin-41* is acting via *lin-29* to control AVM axon regeneration.

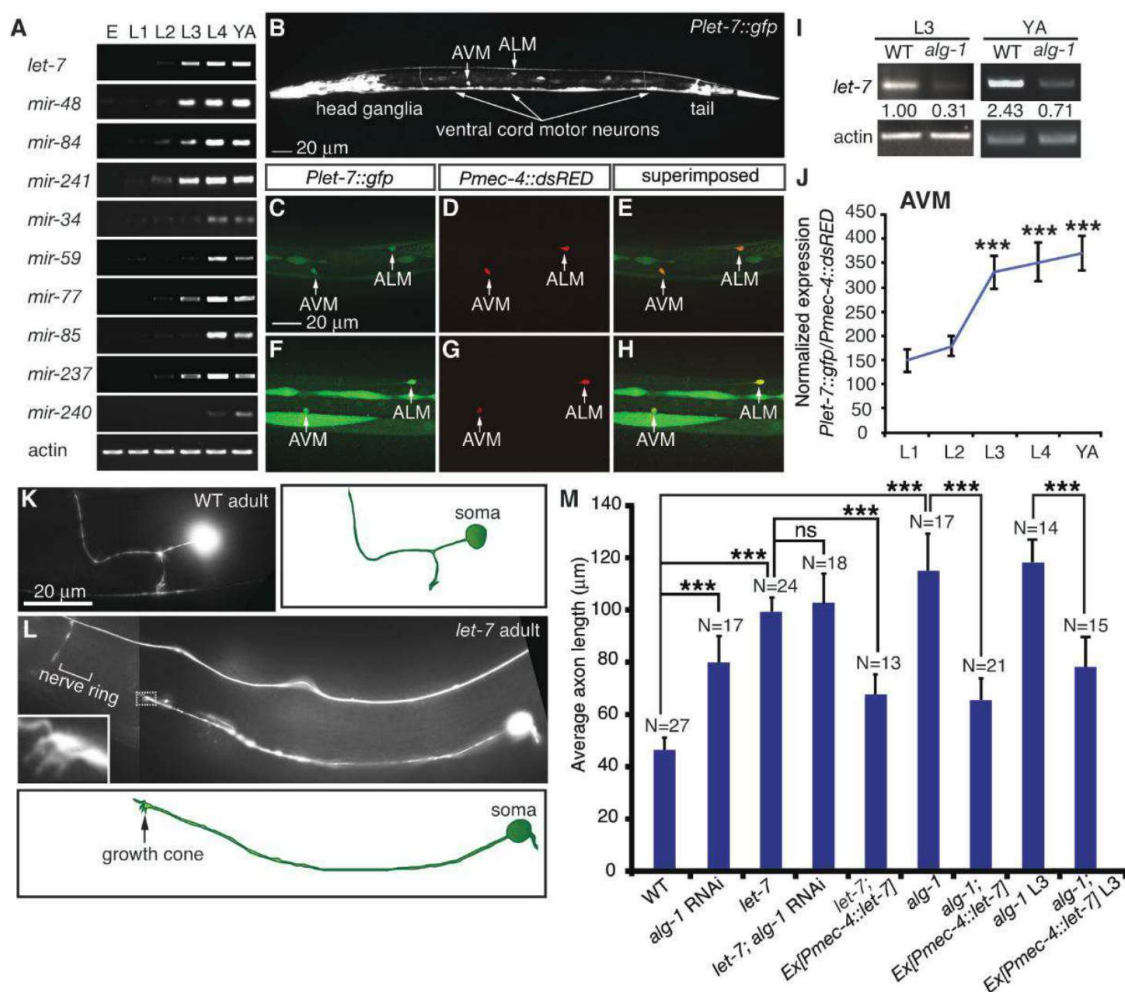
A conserved mitogen-activated protein kinase (MAPK) pathway, including the DLK-1–MAPK kinase kinase and p38 kinase–PMK-3, controls axon regeneration in *C. elegans* (4). This pathway is negatively regulated by the conserved protein RPM-1 (4). We show that *dlk-1* and *pmk-3* mutations suppress the enhanced AVM axon re-

generation of *let-7* mutants, which suggests that *let-7* may act upstream of *dlk-1* and *pmk-3* (fig. S9A). Further, *lin-41* mutations do not suppress the enhanced AVM axon regeneration of *rpm-1* mutants, whereas *pmk-3* and *dlk-1* mutations do, which suggests that *lin-41* may act upstream of or in parallel to *rpm-1* (fig. S9A). Analysis of the RPM-1 level shows no difference between *lin-41* mutants and wild-type animals (fig. S9B), which indicates that either LIN-41 is acting in parallel to RPM-1 or LIN-41 is acting through a posttranslational mechanism to indirectly regulate RPM-1 activity but not its level.

In wild-type animals, mature *let-7* microRNA is expressed at low levels before the L3 stage (Figs. 2A and 4A). In *lin-41* mutants, *let-7* is precociously expressed at the L1 stage (Fig. 4A), which indicates that *lin-41* prevents the early-onset expression of *let-7*. The expression intensity of a *Plet-7::GFP* reporter in the whole animal or in AVM is slightly lower instead of higher in *lin-41* mutants than in wild-type animals at L1 to L3 stages (Fig. 4, B and C), which suggests that *lin-41* inhibits early expression of *let-7* posttranscriptionally.

Recent studies of mouse stem cells showed that mLin41 E3 ubiquitin ligase activity regulates

Fig. 2. The *let-7* expression in AVM and ALM and the *let-7* mutant phenotype in enhancing AVM axon regeneration. (A) Stem-loop RT-PCR analysis of RNA isolated from populations of staged animals revealed late-onset expression of several mature microRNAs. (B) Broad *let-7* expression in neurons at L3. (C to H) The *let-7* promoter drives GFP expression in AVM and ALM touch neurons in L2-stage (C to E) and YA (F to H) animals. The *Pmec-4::dsRED* reporter labels AVM and ALM. (I) Mature *let-7* levels by stem-loop RT-PCR in *alg-1* mutants versus wild-type animals at L3 and YA stages. The value below each lane indicates amount of *let-7* normalized to actin. (J) Average fluorescent intensity of the *Plet-7::GFP* reporter normalized to the *Pmec-4::dsRED* in AVM. Regenerating AVM axons in wild-type adult animals (K) and adult *let-7(n2853)* mutants (L). The dashed box in (L) delineates the growth cone, whose blown up image is shown in the inset. (M) Average AVM axon length. Asterisks indicate cases in which mutants differ from wild-type or a specific comparison is significantly different at ****P* < 0.001.



Argonaute2 turnover (18). Our study shows that the ALG-1 Argonaute is required for *let-7* maturation in *C. elegans* (Fig. 2I). To test whether LIN-41 inhibits the early-onset expression of *let-7* through negative regulation of ALG-1, *alg-1* mutations were examined for their effects on *let-7* expression in *lin-41* mutants. In *lin-41*; *alg-1* double mutants, *let-7* levels were restored to late-onset expression (Fig. 4A). Because only the ectopic (early) expression but not the normal

(late) expression of *let-7* was affected by *alg-1* mutations in *lin-41* mutants, this result suggests that LIN-41 may inhibit ALG-1 only at the early stage to repress early-onset *let-7* expression. Indeed, LIN-41 is no longer able to down-regulate *let-7* expression in the adult stage (Fig. 4D).

Consistent with this interpretation, the level of the ALG-1::GFP fusion protein in AVM at the L1 stage was significantly higher in *lin-41*

mutants than in wild-type animals (Fig. 4E). Using a *lin-41* promoter construct that drives the FLAG-tagged LIN-41 protein expression mainly in neurons (Fig. 3A), we tested whether immunoprecipitation (IP) of ALG-1 would coprecipitate the FLAG-tagged LIN-41 protein. Co-IP of FLAG::LIN-41 with ALG-1 was observed (Fig. 4F and fig. S10A), which suggests that these two proteins may form a complex in neurons. The relatively low LIN-41::FLAG signal

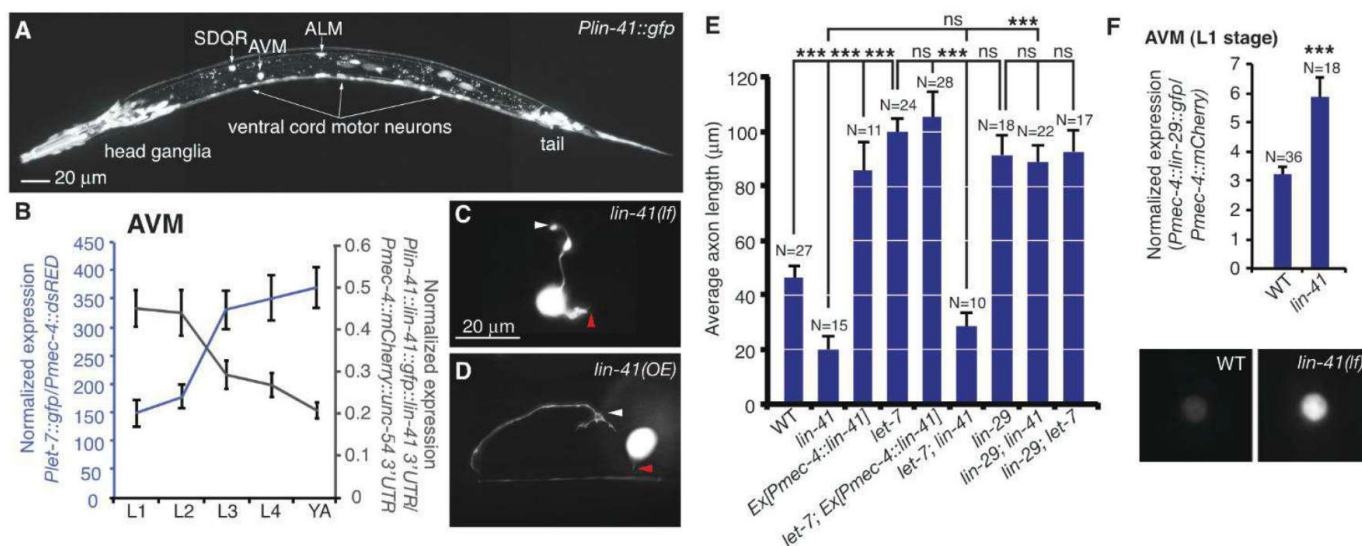


Fig. 3. The *lin-41* expression study and the *lin-41* effect on AVM axon regeneration. (A) Broad *lin-41* expression in neurons at L2. SDQR is an interneuron. (B) The *let-7* promoter reporter and the engineered *lin-41* genomic reporter are temporally regulated in AVM neurons. Typical regenerating AVM axons in adult *lin-41* mutants (C) and *Ex[Pmec-4::lin-41]* transgenic animals (D). Red arrowhead points to the axon break, and white arrowhead

points to the terminus of regenerating axons. Anterior is left, dorsal up. (E) Average AVM axon length. Asterisks indicate cases in which mutants or transgenic animals significantly differ from wild-type or a comparison between mutants is significantly different at $***P < 0.001$. (F) Analysis of the level of the LIN-29::GFP fusion protein in AVM in *lin-41* mutants versus wild-type animals at L1. Images shown are expression of the LIN-29::GFP fusion protein in the AVM nucleus.

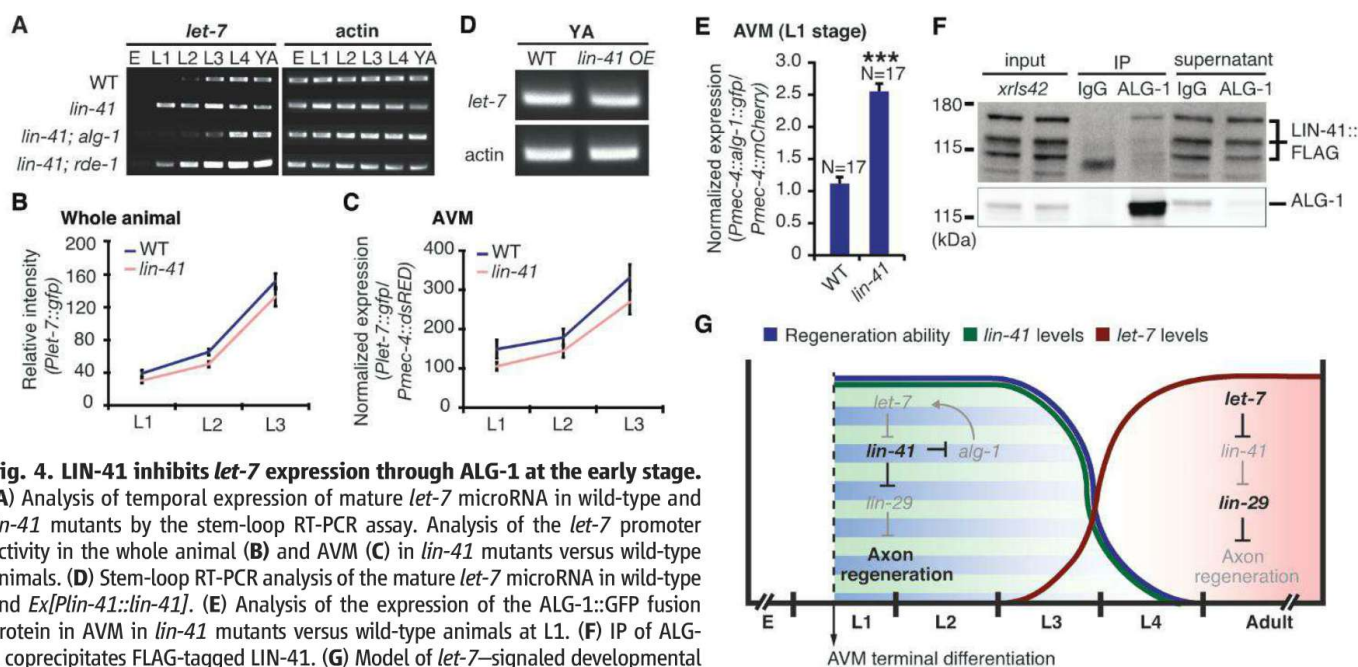


Fig. 4. LIN-41 inhibits *let-7* expression through ALG-1 at the early stage. (A) Analysis of temporal expression of mature *let-7* microRNA in wild-type and *lin-41* mutants by the stem-loop RT-PCR assay. Analysis of the *let-7* promoter activity in the whole animal (B) and AVM (C) in *lin-41* mutants versus wild-type animals. (D) Stem-loop RT-PCR analysis of the mature *let-7* microRNA in wild-type and *Ex[Plin-41::lin-41]*. (E) Analysis of the expression of the ALG-1::GFP fusion protein in AVM in *lin-41* mutants versus wild-type animals at L1. (F) IP of ALG-1 coprecipitates FLAG-tagged LIN-41. (G) Model of *let-7*-signaled developmental decline in AVM axon regeneration.

intensity in the ALG-1 IP suggests that only a subpopulation of LIN-41 is in complexes with ALG-1. Similarly, IP of GFP-tagged LIN-41 pulled down a subset of the ALG-1 protein population (fig. S10B). IP of small subsets of protein populations with components of microRNA-induced silencing complex has precedents (15, 19).

Even though *let-7* levels decline significantly by day 4 into the adult stage (fig. S11, A and B), AVM axon regeneration remained significantly enhanced in aged animals that either overexpress *lin-41* or contain *let-7* mutations (fig. S11, C and D), which indicates that the *let-7/lin-41* pathway regulates axon regeneration even in aged AVM. Furthermore, *let-7* mutations significantly enhance, whereas *lin-41* mutations significantly reduce, axon regeneration in several neurons that coexpress both genes, which suggests that the role of *let-7/lin-41* in regulating axon regeneration can be extended beyond AVM neurons (fig. S12).

In this study, we show that *let-7* contributes to a developmental decline in AVM axon regeneration. We identify LIN-41 as an important AVM axon regeneration-promoting factor. *let-7* represses *lin-41* expression to inhibit AVM axon regeneration in older neurons. Our results suggest a negative regulatory loop between *let-7*

and *lin-41* (Fig. 4G). In younger neurons, *lin-41* inhibits *let-7* expression through negative regulation of ALG-1. In older neurons, up-regulation of *let-7* overcomes the *lin-41* inhibition and, in turn, represses the *lin-41* expression through the *lin-41* 3' UTR. Like *C. elegans*, mammals also exhibit a developmental decline in axon regeneration. Our findings suggest that it may be possible to enhance axon regeneration after injury through therapeutic inhibition of the *let-7* microRNA in neurons and, thereby, to restore their youthful regenerative capacity.

References and Notes

1. H. Chiu, A. Alqadah, C.-F. Chuang, C. Chang, *Cell Adhes. Migr.* **5**, 387 (2011).
2. Z. Wu et al., *Proc. Natl. Acad. Sci. U.S.A.* **104**, 15132 (2007).
3. C. V. Gabel, F. Antoine, C.-F. Chuang, A. D. T. Samuel, C. Chang, *Development* **135**, 3623 (2008).
4. M. Hammarlund, P. Nix, L. Hauth, E. M. Jorgensen, M. Bastiani, *Science* **323**, 802 (2009).
5. J. L. Goldberg, M. P. Klassen, Y. Hua, B. A. Barres, *Science* **296**, 1860 (2002).
6. M. Boehm, F. Slack, *Science* **310**, 1954 (2005).
7. R. C. Lee, R. L. Feinbaum, V. Ambros, *Cell* **75**, 843 (1993).
8. B. Wightman, I. Ha, G. Ruvkun, *Cell* **75**, 855 (1993).
9. A. E. Pasquinelli et al., *Nature* **408**, 86 (2000).
10. F. J. Slack et al., *Mol. Cell* **5**, 659 (2000).
11. R. J. Johnston Jr., O. Hobert, *Nature* **426**, 845 (2003).

12. S. Chang, R. J. Johnston Jr., C. Frøkjær-Jensen, S. Lockery, O. Hobert, *Nature* **430**, 785 (2004).
13. Y.-W. Hsieh, C. Chang, C.-F. Chuang, *PLoS Genet.* **8**, e1002864 (2012).
14. Y. Zou, H. Chiu, D. Domenger, C.-F. Chuang, C. Chang, *Sci. Signal.* **5**, ra43 (2012).
15. T. F. Duchaine et al., *Cell* **124**, 343 (2006).
16. S. Y. Lin et al., *Dev. Cell* **4**, 639 (2003).
17. A. E. Rougvie, V. Ambros, *Development* **121**, 2491 (1995).
18. A. Rybak et al., *Nat. Cell Biol.* **11**, 1411 (2009).
19. C. M. Hammell, I. Lubin, P. R. Boag, T. K. Blackwell, V. Ambros, *Cell* **136**, 926 (2009).

Acknowledgments: We thank the *C. elegans* Genetic Center for *C. elegans* strains; the WormBase for readily accessible information; F. Ciamacco, B. Bayne, and C. Carrelli for technical assistance; A. Fire for *C. elegans* vectors; and V. Cleghon for critical reading of the manuscript. This work was funded by grants from the Whitehall Foundation (C.C. and C.-F.C.), the March of Dimes Foundation (C.C.), the Canada Foundation for Innovation (C.C.), by Alfred P. Sloan Research Fellowships to C.-F.C., by NIH grant R01 GM098026 to C.-F.C., and by NIH grant R01 GM034028-25 to V.A.

Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6130/372/DC1

Materials and Methods

Figs. S1 to S12

Table S1

References (20–23)

10 October 2012; accepted 8 March 2013

10.1126/science.1231321

A Neural Marker of Perceptual Consciousness in Infants

Sid Kouider,^{1,2*} Carsten Stahlhut,² Sofie V. Gelskov,^{1,3} Leonardo S. Barbosa,¹ Michel Dutat,¹ Vincent de Gardelle,¹ Anne Christophe,¹ Stanislas Dehaene,^{4,5,6,7} Ghislaine Dehaene-Lambertz^{5,6,7}

Infants have a sophisticated behavioral and cognitive repertoire suggestive of a capacity for conscious reflection. Yet, demonstrating conscious access in infants remains challenging, mainly because they cannot report their thoughts. Here, to circumvent this problem, we studied whether an electrophysiological signature of consciousness found in adults, corresponding to a late nonlinear cortical response [~300 milliseconds (ms)] to brief pictures, already exists in infants. We recorded event-related potentials while 5-, 12-, and 15-month-old infants ($N = 80$) viewed masked faces at various levels of visibility. In all age groups, we found a late slow wave showing a nonlinear profile at the expected perceptual thresholds. However, this late component shifted from a weak and delayed response in 5-month-olds (starting around 900 ms) to a more sustained and faster response in older infants (around 750 ms). These results reveal that the brain mechanisms underlying the threshold for conscious perception are already present in infancy but undergo a slow acceleration during development.

Recent research shows that, in the first year of life, preverbal infants already display an impressive array of cognitive competences. For instance, their eye movements betray a capacity to monitor other people's beliefs at 7 months (1) and to draw probabilistic predictions about visual scenes at 12 months (2). Given these complex behaviors, one might consider it obvious that infants already have a conscious experience of their environment. However, this conclusion is unwarranted, because the presence of unconscious priming and "blind-

sight" behaviors in normal and impaired adults (3, 4) shows that sophisticated processing can occur without consciousness.

How, then, might one test whether the brain mechanisms for conscious access are already present in infancy? Studying consciousness and its neural correlates in adults requires the collection of subjective reports of experience, classically through psychophysical paradigms contrasting visible and invisible stimuli (5). It is not impossible to obtain subjective reports from nonverbal organisms. For instance, monkeys can be trained

to report the presence or absence of a stimulus, either by touching the location of a stimulus on a screen or by touching an alternative key to indicate that no stimulus had been presented. After a unilateral lesion in V1, they consistently press the "absent" key for stimuli contralateral to the lesioned side, although they remain able to localize them with high accuracy, which suggests that they undergo a "blindsight" phenomenon similar to that of human patients (6). However, it seems much more difficult to train infants to report similarly about their thoughts and percepts, which renders the issue of infant consciousness particularly challenging.

In this study, we follow an alternative strategy: examining whether the neural signatures of perceptual consciousness that are observed in adults can already be obtained in the developing brain. We capitalize on visual masking, a psychophysical phenomenon whereby a brief display, when followed by a second picture, vanishes from awareness. Previous research in adults has shown that the perception of masked displays

¹Laboratoire de Sciences Cognitives et Psycholinguistique, EHESS/CNRS/ENS-DEC, 75005 Paris, France. ²Section for Cognitive Systems, Department of Informatics and Mathematical Modeling, Technical University of Denmark, 2800 Kongens Lyngby, Denmark. ³Danish Research Center for Magnetic Resonance, Copenhagen University Hospital, 2650 Hvidovre, Denmark. ⁴Collège de France, 75231 Paris, France. ⁵INSERM, U992, Cognitive Neuroimaging Unit, 91191 Gif-sur-Yvette, France. ⁶CEA, NeuroSpin Center, 91191 Gif-sur-Yvette, France. ⁷Université Paris XI, 91405 Orsay, France.

*Corresponding author. E-mail: sid.kouider@ens.fr

follows a two-stage process with distinct electrophysiological signatures (7–10). During the first ~200 to 300 ms of processing, brain re-

sponses increase linearly with the stimulus energy or duration. This early linear stage can be observed even on subliminal trials in which the

stimulus is subjectively invisible. By contrast, the second stage, which starts after ~300 ms, is characterized by a nonlinear, essentially all-or-none change in brain activity detectable with event-related potentials (ERPs) (8, 9, 11) and intracranial recordings (12). Note that this second stage occurs specifically on trials reported as consciously seen [e.g., (5, 13, 14)]. During this stage, even a brief external stimulation, if it exceeds a certain threshold, can lead to a large and long-lasting pattern of activity involving the recruitment of a distributed parieto-frontal network, as well as the reactivation of the initial sensory regions. This sustained activity allows for the maintenance of the perceptual representation long after the stimulus is gone and coincides with subjective reports of visibility (8, 9).

Here, we examined whether the electrophysiological signatures of this two-stage process can be observed in infants. The presence of both early linear and late nonlinear electrophysiological markers during visual perception in infants would indicate that, first, the same architecture for perception is already at work at early stages of development and, second, that a distinction between nonconscious and conscious events may already be delineated in infants. We recorded high-density (128 electrodes) ERPs in three groups of infants [5-month-olds ($n = 30$), 12-month-olds ($n = 29$), and 15-month-olds ($n = 21$)] while they looked at masked faces presented at various durations, so as to induce different levels of visibility [see Fig. 1A and (15) for details].

Using the same stimuli in a behavioral study of preferential looking, we recently measured the masking threshold in infancy and evidenced important changes occurring at the end of the first year of life (16). Although 5- and 10-month-old infants oriented their gaze only to faces presented for 150 ms or longer, 15-month-olds exhibited a perceptual threshold closer to the adult value (between 50 and 100 ms). Furthermore, whereas 5- and 10-month-old infants oriented to supra-threshold faces in a purely reactive manner (i.e., performance systematically came back to the level of chance in between face occurrences, even though they were repeatedly presented at the same peripheral location on each trial), 15-month-olds anticipated the face reappearance, which demonstrated that they were not only

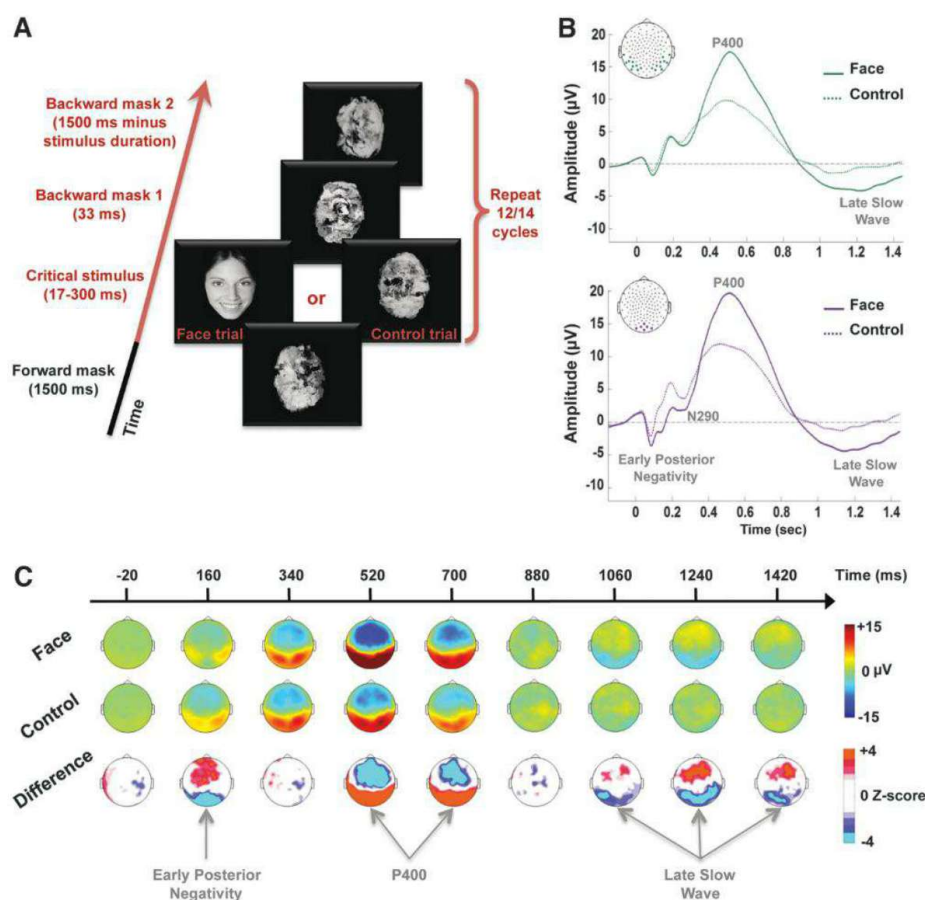
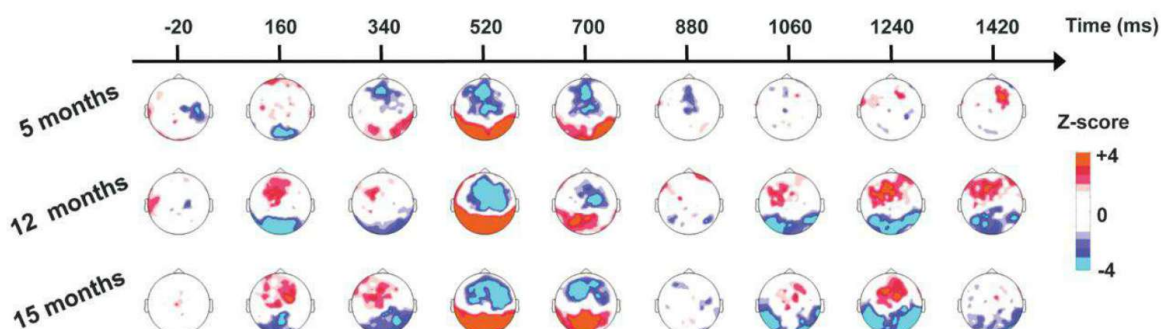


Fig. 1. (A) Schematic description of the procedure. Visibility was manipulated by presenting faces at various durations, within a series of masking patterns (scrambled layers of upside-down faces and objects) that prevented visual persistence and lingering afterimages. A control condition comprising exclusively scrambled patterns allowed for the localization of face-sensitive components. The younger (5-month-old) infants were tested with durations ranging from 50 to 300 ms in steps of 50 ms. The older (12- and 15-month-old) groups were tested with face durations of 17, 33, 50, 100, 150, 200, and 250 ms. Infants received blocks with a forward mask followed by the random presentation of face and control trials at each duration, leading to 12 cycles in younger and 14 cycles in older infants. **(B and C) Analysis of global ERPs** (collapsing across the five durations common to all infants; i.e., from 50 to 250 ms). **(B)** Grand-average responses recorded over posterior medial occipital and lateral occipitotemporal electrodes and revealing the four components responding preferably to faces compared with scrambled controls (early posterior negativity, N290, P400, and LSW). **(C)** Two-dimensional (2D) scalp topographies over time showing the voltage ERP evoked by face and control trials, and their difference in terms of statistical significance maps (Z-scores).

Fig. 2. Event-related potentials as a function of age. Over time, 2D scalp topographies show statistical significance maps (Z-scores) of the face-control difference at 5, 12, and 15 months of age.



able to maintain the perceptual representation of a stimulus over time but also to use this information to adapt their behavior. At the brain level, we thus predicted a marked change around the end of the first year of life in infants. Specifically, we expected that the transition from linear brain responses to a sudden, nonlinear increase (a neural marker which in adults indicates the maintenance of perceptual information for conscious report) would be more pronounced and occur at shorter stimulus durations in the older babies.

We first extracted the event-related brain potentials evoked by masked faces relative to scrambled control stimuli. This first step allowed us to identify face-sensitive latencies and clusters of electrodes independently of infants' age and stimulus duration. Although faces were very briefly presented and embedded in masks, we observed the classical ERP components of face perception in infants, with a series of four components responding preferably to faces (Fig. 1, B and C). The first difference was an early posterior negativity (EPN) peaking around 150 ms [$t(79) = 6.38, P < 0.001$] over medial occipital electrodes. This component is likely to reflect the earliest bottom-up stage, where structured images are separated from scrambled stimuli (17). It was followed by two components classically associated with face processing in infants (18, 19): an N290 over the same posterior occipital electrodes [$t(79) = 2.35, P < 0.05$] and then a P400 extending bilaterally from occipital to temporal regions [$t(79) = 11.29, P < 0.001$]. Although the homology of these components with the N170 specifically elicited by faces in adults remains debated, the N290 is sensitive to the presence of contrasted human eyes, whereas the P400 depends on infants' knowledge of the prototypical face configuration (18, 19). Finally, we observed a sustained late negativity starting from 900 ms over the same occipito-temporal electrodes, along with a positivity over anterior

electrodes [$t(79) = 4.75, P < 0.001$]. This late response corresponds to the late slow wave (LSW), which has been linked to stimulus encoding and recognition memory but also more generally to attention and novelty detection (18–21).

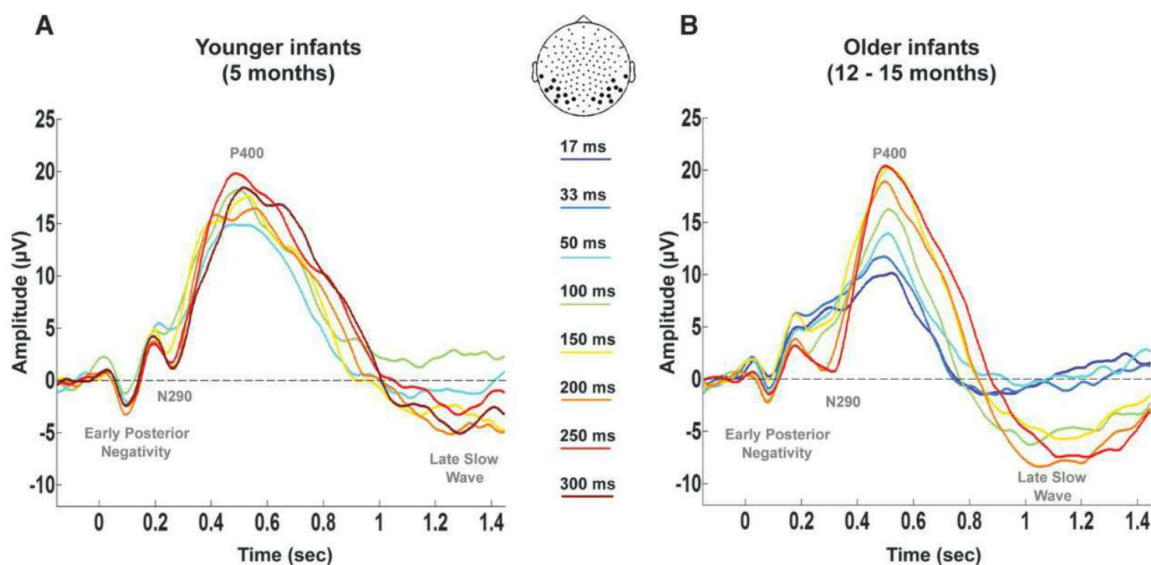
We then examined how these components evolved with age (Fig. 2 and figs. S1 to S3). The EPN and P400 components were significant in all age groups (all P values were <0.01) and were of equivalent magnitude across the three age groups (both F values were <1) (see fig. S1). By contrast, we found robust interactions with age for the N290 ($F_{2,77} = 10.17, P < 0.001$) and for the LSW ($F_{2,77} = 4.17, P = 0.019$), which suggested the presence of a developmental change for these two components. Indeed, for both components, the difference between faces and controls occurred solely in 12-month-olds (both P values were <0.001) and 15-month-olds (both P values were <0.005), with the same amplitude for both groups ($F < 1$).

Having established which components were sensitive to faces, we studied their variation as a function of stimulus duration in the face trials. Because the results for the groups of 12- and 15-month-olds were virtually identical, they were collapsed to increase signal-to-noise ratio. The waveforms elicited by faces at each stimulus duration are shown in Fig. 3 (see fig. S4 for control stimuli and difference waves). In the older group of 12- to 15-month-olds, it is apparent that the N290, P400, and LSW components are strongly and monotonically modulated by stimulus duration. Furthermore, the LSW is essentially absent for face presentations of up to 50 ms duration and suddenly jumps to a strong negativity for durations of 100 ms and beyond. This is consistent with our previous behavioral study, which suggests a perceptual threshold around 50 to 100 ms of presentation duration (16). To evaluate these points statistically, we submitted each electrode and time point of the face conditions to two contrasts (8). The first was a linear contrast eval-

uating a continuous variation in voltage with stimulus duration. The second was nonlinear and tested for a jump around the presumed threshold, i.e., a larger increase in voltage when presentation duration moved from 50 to 100 ms, compared with the other ranges of stimulus duration (average change in voltage from 16 to 50 ms and from 100 to 250 ms). The results confirmed the presence of both linear and nonlinear effects over the same occipito-temporal electrodes, but with distinct time courses (Fig. 4). Although the linear effect became significant relatively early at 180 ms, the nonlinear trend emerged rather abruptly only at a later stage, around 750 ms (Fig. 4, C and D). Furthermore, whereas the N290 and P400 increased in a strictly linear manner, both linear and nonlinear contrasts were significant for the LSW. This joint significance was due to a sigmoidal response profile: The LSW showed a general increase with stimulus duration, but with a large nonlinear jump around the previously identified perceptual threshold (from 50 to 100 ms). These results, which dissociate early linear and late nonlinear components, demonstrate that an adultlike, although much slower, sequence of perceptual stages is already observed by the end of the first year of life.

What about younger infants? In comparison with the older group, modulations of voltage by face duration were overall smaller in 5-month-olds (Fig. 3A). Nevertheless, the linear contrast again achieved significance for the N290, the P400, and even for the LSW (Fig. 4, A to C). Although no significant LSW difference wave was found at this age in the global analysis (i.e., collapsing stimulus durations) (see Fig. 2 and text above), an LSW component was actually present and differed significantly from baseline at face durations of 150 ms and above (all P values were <0.002) (Fig. 3A). Again, this is consistent with our previous behavioral study showing that 5-month-old infants did not orient to faces presented for duration of 100 ms or less

Fig. 3. (A and B) Variation of ERPs with stimulus duration in younger and older infants. Graphs show the grand-average responses recorded over occipitotemporal electrodes for faces as a function of stimulus duration.



but showed an abrupt increase in responding to faces from 100 to 150 ms (16). Indeed, our contrast testing for a nonlinear response component occurring between the predicted durations of 100 ms and 150 ms began to deviate from baseline abruptly around 900 ms, simultaneously with the LSW (Fig. 4, B to D). However, we observed that the LSW component was slow and small at this age, and statistical significance of the nonlinearity only attained in the latest period of the epoch (starting at 1320 ms). Thus, we found that the two-stage architecture for perception can be evidenced early on during development, in infants as young as 5 months, although this distinction was not as clearly delineated as for older infants.

Because the LSW exhibits a late nonlinearity that matches the visibility thresholds established in previous behavioral testing (16), we suggest that this electrophysiological component constitutes a reliable neural index of conscious access to perceptual information in preverbal infants. Note that this late component cannot be ascribed to unmatched number of trials or larger movement artifacts in one specific condition [see (15) for details]. LSWs, generally recorded after 600 ms, have been first related to memory encoding and recognition (18, 19). Since then, slow waves have been described in different

visual and auditory paradigms in awake and attentive infants (20, 21). For instance, the LSW is observed after a deviant sound in awake, but not asleep, infants (22). It also reflects the infant's allocation of attention toward the mother's face, a familiar toy, or a novel event against a background of familiar or partially familiar events (18, 20). The diversity of experimental paradigms eliciting LSWs in infants suggests that this component reflects a generic amodal system involving higher-order processes, such as attention and working memory. Because of these functional characteristics, equivalence with the P300 in adults has been proposed (23). Our results support this interpretation by showing that the LSW exhibits the same nonlinear response profile as the P300 and follows earlier components responding in a linear fashion as a function of stimulus intensity (8, 11). Note that the adult P300 component strongly correlates with subjective reports of visibility (5, 8, 9) and is therefore thought to constitute a neural signature of access to consciousness. The functional similarity of infant and adult responses, combined with our previous behavioral results (16), suggests that a stage of conscious processing already exists in infants and that the LSW constitutes a reliable neural signature of consciousness in infants.

Nonetheless, our results also reveal that this neural marker of consciousness in infants is triggered at a much later time than in adults. Indeed, whereas the nonlinear response to masked stimuli is found around 300 ms in the adult brain (8, 11), here, we observed it around 750 ms in 12- to 15-month-old infants, and after 900 ms or more in 5-month-olds. Hence, this second processing stage is disproportionately slow in infants and remains so for many months.

What constitutes the neural basis of this exceedingly slow response? The first stages of perception (indexed by the EPN and P400 components), which exhibited equivalent latencies in all age groups, probably reflect the rapid maturation of the sensory visual system during the first few months of life (24). By contrast, the long latencies of the LSW are compatible with the notion that this nonlinear response depends on a large-scale distributed network of long-distance corticocortical connections (5). Most of these long-fiber tracts are present in infants (25) and even fetuses (26), but they remain weakly myelinated through the first year of life (24). Such a weak myelination could explain why the LSW emerged later in 5-month-olds compared with older infants. It would lead to a reduced and slower interplay between sensory regions and associative parietal-prefrontal

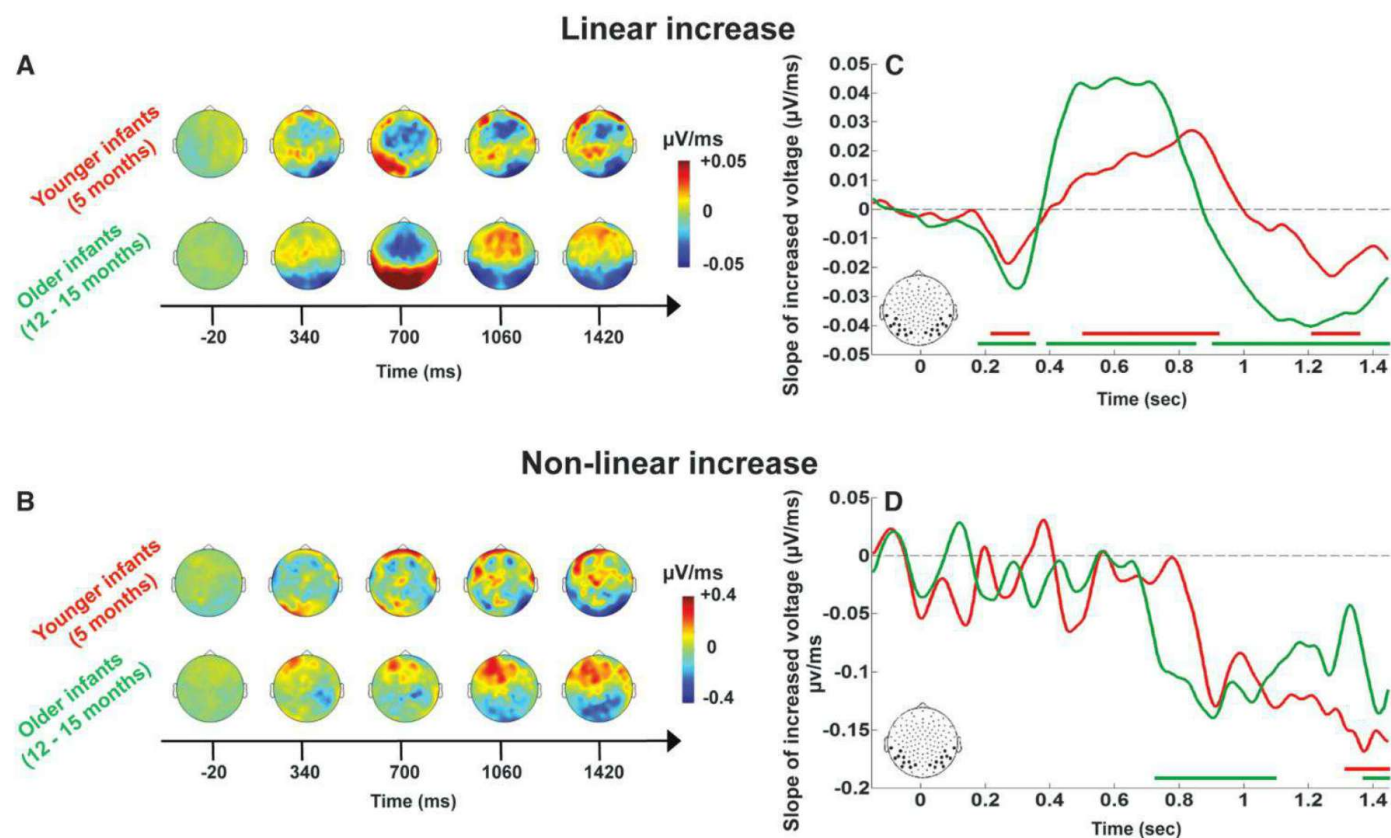


Fig. 4. (A to D) Time course of the linear and nonlinear increases in brain activity as a function of face duration, in younger and older infants. The 2D plots show the scalp topographies of the linear and nonlinear contrasts. The unit of measurement is the slope with which voltage increases

with stimulus duration, expressed in units of microvolts per millisecond. Response profiles over occipitotemporal electrodes are accompanied with horizontal bars indicating temporal clusters of 10 consecutive significant time points (40 ms).

areas, which limits the functional connectivity that is necessary for the maintenance and flexible use of sensory information. Consistent with this interpretation, the LSW scalp topography, showing posterior negativities over bilateral occipitotemporal regions, is suggestive of a late reactivation of sensory regions. In adults, such a late reactivation has been argued to reflect top-down mechanisms of attentional amplification that allow for the maintenance of a stimulus representation over time that is characteristic of perceptual consciousness (5, 7, 9). Another potential factor is the protracted dendritic development and synaptogenesis in associative areas (27). Indeed, the prefrontal cortex shows a marked maturation and massive reorganization at the end of the first year of life, resulting in dramatic improvements in a wide range of cognitive abilities at that age (28). In any case, the acceleration of information transfer between associative and sensory areas might be a key factor underlying infants' improved perceptual efficiency across development.

In sum, our data indicate that infant perception is organized into a series of stages similarly to adult perception; these include, crucially, a late nonlinear stage that, in adults, systematically accompanies reports of conscious perception and, in infants, correlates with psychophysical thresholds for orienting to masked stimuli. We propose that this late nonlinear response constitutes a new, specific, and objectively measurable candidate marker that putatively reflects conscious perception. It is important to acknowledge that our research does not provide a direct proof of subjective experience. Indeed, it is a genuine philosophical problem whether such a proof can ever be obtained from purely objec-

tive neurophysiological data. Rather, we show that neural markers of consciousness found in adults can be generalized to infant populations. Such objective measures have proven useful to probe consciousness in patients in a vegetative state and in minimally conscious patients (29) and might help pediatricians confront issues of infant consciousness in relation to anesthesia, pain, and pathologies.

References and Notes

1. A. M. Kovács, E. Téglás, A. D. Endress, *Science* **330**, 1830 (2010).
2. E. Téglás et al., *Science* **332**, 1054 (2011).
3. S. Kouider, S. Dehaene, *Philos. Trans. R. Soc. London B Biol. Sci.* **362**, 857 (2007).
4. L. Weiskrantz, *Consciousness Lost and Found: A Neuropsychological Exploration* (Oxford Univ. Press, New York, 1997).
5. S. Dehaene, J. P. Changeux, *Neuron* **70**, 200 (2011).
6. A. Cowey, P. Stoerig, *Nature* **373**, 247 (1995).
7. V. A. Lamme, P. R. Roelfsema, *Trends Neurosci.* **23**, 571 (2000).
8. A. Del Cul, S. Baillet, S. Dehaene, *PLoS Biol.* **5**, e260 (2007).
9. C. Sergent, S. Baillet, S. Dehaene, *Nat. Neurosci.* **8**, 1391 (2005).
10. M. M. Chun, M. C. Potter, *J. Exp. Psychol. Hum. Percept. Perform.* **21**, 109 (1995).
11. L. Melloni et al., *J. Neurosci.* **27**, 2858 (2007).
12. R. Q. Quiroga, R. Mukamel, E. A. Isham, R. Malach, I. Fried, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 3599 (2008).
13. E. K. Vogel, S. J. Luck, K. L. Shapiro, *J. Exp. Psychol. Hum. Percept. Perform.* **24**, 1656 (1998).
14. S. Kouider, S. Dehaene, in *Mental Processes in the Human Brain*, J. Driver, P. Haggard, T. Shallice, Eds. (Oxford Univ. Press, Oxford, 2007), pp. 155–185.
15. Materials and methods are available as supplementary materials on Science Online.
16. S. V. Gelskov, S. Kouider, *Cognition* **114**, 285 (2010).
17. B. Rossion, S. Caharel, *Vision Res.* **51**, 1297 (2011).
18. M. de Haan, C. A. Nelson, *Dev. Psychol.* **35**, 1113 (1999).
19. M. de Haan, M. H. Johnson, H. Halit, *Int. J. Psychophysiol.* **51**, 45 (2003).
20. G. D. Reynolds, J. E. Richards, *Dev. Psychol.* **41**, 598 (2005).
21. G. Csibra, E. Kushnerenko, T. Grossmann, in *Handbook of Developmental Cognitive Neuroscience*, C. A. Nelson and M. Luciana, Eds. (MIT Press, Cambridge, MA, ed. 2, 2008), pp. 247–262.
22. A. D. Friederici, M. Friedrich, C. Weber, *Neuroreport* **13**, 1251 (2002).
23. C. A. Nelson, K. M. Thomas, M. de Haan, S. S. Wewerka, *Int. J. Psychophysiol.* **29**, 145 (1998).
24. P. Yakovlev, A. R. Lecours, in *Regional Development of the Brain in Early Life*, A. Minkowski, Ed. (Blackwell, Oxford, 1967), pp. 3–69.
25. J. Dubois, L. Hertz-Pannier, G. Dehaene-Lambertz, Y. Cointepas, D. Le Bihan, *Neuroimage* **30**, 1121 (2006).
26. E. Takahashi, R. D. Folkerth, A. M. Galaburda, P. E. Grant, *Cereb. Cortex* **22**, 455 (2012).
27. M. J. Koenderink, H. B. Uylings, *Brain Res.* **678**, 233 (1995).
28. A. Diamond, P. S. Goldman-Rakic, *Exp. Brain Res.* **74**, 24 (1989).
29. T. A. Bekinschtein et al., *Proc. Natl. Acad. Sci. U.S.A.* **106**, 1672 (2009).

Acknowledgments: This research was supported by funding from the Agence National de la Recherche and from the European Research Council ("DynaMind" project) to S.K., from the Lundbeck Foundation (CIMBI project) to C.S. and from the McDonnell foundation to G.D.-L. We thank C. Summerfield for suggestions on the manuscript; A.-C. Fievet, and I. Vendelin for help with the data collection; and C. Billard (CHU Kremlin-Bicêtre), D. Chabrol (Hôpital Cochin), and L. K. Hansen (Danish Technical University) for their support to our research.

Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6130/376/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S5
References (30–32)

8 November 2012; accepted 19 February 2013
10.1126/science.1232509

Support the sciences. **Get rewarded.**

Show your AAAS pride and reward yourself with the new AAAS Platinum Advantage Rewards Card from NASA Federal Credit Union.

Apply now and get
10,000 bonus points!

Go to nasafcu.com/AAASpromo

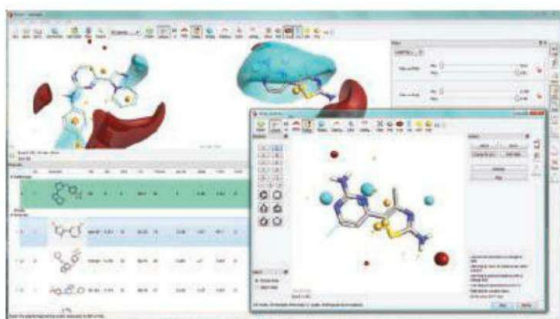


Get **10,000 bonus points** if you sign up for a card and spend \$3,000 within 90 days of account opening.

Learn more at
nasafcu.com/AAASpromo.

Subject to credit approval.
Membership in AAAS and NASA FCU is required.
NASA FCU is federally insured by NCUA.





MOLECULE DESIGN

torchV10 is a complete desktop molecule design and 3-D SAR tool for medicinal chemists. torchV10 uses molecular fields to show the binding patterns of compounds. This protein's eye view gives clear insights into the causes of biological activity. Working with torchV10, medicinal chemists can see how to optimize the shape and electrostatic properties of their series, and rapidly identify the best next molecule to synthesize. torchV10 is a powerful molecule design and 3-D SAR tool for medicinal chemists. Use it to take leaps in compound design while maintaining or improving biological activity. Compare actives and inactives from multiple series, gathering the best from each. Employ this knowledge in the next design iteration using the in-built molecular editor for immediate feedback.

Cresset Group

For info: +44-(0)-1707-356120 | www.cresset-group.com

HUMAN RNA SEQUENCING DATA ANALYSIS

The Ingenuity iReport now supports analysis and interpretation of human isoform-level RNA sequencing data. Ingenuity iReport for Isoform-level Human RNA-Seq data enables researchers to quickly and easily identify significantly regulated isoforms, interpreting the impact of those expression changes in the context of the pathways, phenotypes, and molecular interactions. Life science researchers are rapidly adopting RNA-Seq methods for whole transcriptome analysis and identification of differentially expressed isoforms. However, data analysis and interpretation remains a challenge for researchers who find themselves faced with large, complex data sets and limited ability to generate biological insights quickly. The introduction of isoform support in iReport allows bench scientists to directly visualize and translate expression changes for individual isoforms into meaningful insights in a user-friendly, web-based analysis application.

Ingenuity

For info: 650-381-5056 | www.ingenuity.com/getireport

REAL-TIME SUPER-RESOLUTION SYSTEM

The MetaMorph Super-Resolution System with synchronized image acquisition and processing enables analysis of object details smaller than 250 nm in fixed and live cells. The new system features real-time image processing with its GPU accelerated hardware, expanding the capabilities of optical microscopy in research and supports numerous scientific applications, from time-lapse studies to 3-D investigations. Using light microscopy alone, images of biological matter smaller than 250 nm appear blurred and, as a result, are virtually impossible to analyze. Super-resolution microscopy can help to overcome the limits of light microscopy by reconstructing images at a resolution surpassing the diffraction limit of the objective lens. With novel image processing techniques, the new MetaMorph Super-Resolution System is capable of 20 nm lateral resolution in real-time. The user-friendly software guides researchers through setup, acquisition, and analysis, with a dedicated workspace in the user interface ensuring that relevant configuration and display settings are readily accessible.

Molecular Devices

For info: 800-635-5577 | www.moleculardevices.com

SAMPLE TRACKING

The new VisionTracker database software is designed for more accurate and efficient management, tracking, and retrieval of laboratory samples. VisionTracker software provides a robust and secure database to organize and track sample information and storage location. The VisionTracker database has an intuitive graphical interface and comes preloaded with options for Thermo Scientific Cryo and 2-D barcoded storage tubes and boxes, as well as Thermo Scientific freezers, allowing quick and easy integration into any storage workflow or laboratory environment. In addition, VisionTracker is ideal for use with Thermo Scientific VisionMate 2-D barcode readers to simplify management and tracking of barcoded samples. Featuring an intuitive interface, VisionTracker software enables easy searches including sample addition, mother-to-daughter aliquoting, and sample disposition. Additional security features include audit trails of all sample data with a date/time stamp as well as password-protected access control.

Thermo Fisher Scientific

For info: 800-345-0206 | www.thermoscientific.com/visiontracker

NEXT GENERATION SEQUENCING ANALYSIS

The latest version (2.3.2) of the NGS Analysis software package, NextGENe, includes several additional application modules as well as customer requested tools designed to increase the quality and speed of their NGS analysis workflow. New applications included in the updated version include Copy Number Variation (CNV), HLA Analysis as well as a Somatic Mutation mining tool for use with all Next Generation Sequencing platforms including those available from Ion Torrent, Illumina, and Roche. New functions and capabilities integrated into this NextGENe software version include compatibility with the new Ion Torrent Suite 3.4 data format, ability to report annotation relative to multiple transcripts, more effective tools for the support of paired reads and removal of duplicate reads from analysis as well as many tools to increase the quality and speed of NGS sequence analysis with NextGENe software. NextGENe is a comprehensive, freestanding Windows-based analysis program for all NGS sequencing platforms.

Soft Genetics

For info: 888-791-1270 | www.softgenetics.com

Electronically submit your new product description or product literature information! Go to www.sciencemag.org/products/newproducts.dtl for more information.

Newly offered instrumentation, apparatus, and laboratory materials of interest to researchers in all disciplines in academic, industrial, and governmental organizations are featured in this space. Emphasis is given to purpose, chief characteristics, and availability of products and materials. Endorsement by Science or AAAS of any products or materials mentioned is not implied. Additional information may be obtained from the manufacturer or supplier.

A large, detailed portrait of Sir Isaac Newton, showing him with long, curly hair and a serious expression, wearing a dark coat over a white shirt.

Science

There's only one
SIR ISAAC NEWTON

Sir Isaac Newton's contribution to science can only be described as unique. Over his lifetime, Newton offered insights into physics, mathematics, natural philosophy, and even alchemy, and is now considered by many to be one of the greatest scientists who ever lived. In 1687, the publication of his *Philosophiæ Naturalis Principia Mathematica* was an influential landmark in scientific thinking that defined the principles of universal gravitation and the laws of motion—setting the foundation that scientists would turn to for over 300 years.

Today, scientists from around the world turn to *Science*. With 700,000 print readers every week and 3.6 million unique visitors to the online site each month, *Science* reaches more people than any other scientific print publication or website. What's more, as part of the non-profit AAAS, the revenue generated by *Science* supports programs around the world that help inform science policymakers, enhance science diplomacy, strengthen the scientific workforce, and improve science education.

So if you want to reach physicists, mathematicians, life scientists, or even the occasional alchemist, there's only one *Science*. Visit *Science* today at sciencemag.org.



AAAS

For your advertising needs, there's only one **Science**

sciencemag.org



Science Careers Advertising

For full advertising details, go to ScienceCareers.org and click For Employers, or call one of our representatives.

Tracy Holmes
Worldwide Associate Director
Science Careers
Phone: +44 (0) 1223 326525

THE AMERICAS

E-mail: advertise@sciencecareers.org
Fax: 202-289-6742

Tina Burks
East Coast/West Coast/South America
Phone: 202-326-6577

Marci Gallun
Midwest/Canada
Phone: 202-326-6582

Candice Nulsen
Corporate
Phone: 202-256-1528

Online Job Posting Questions
Phone: 202-312-6375

EUROPE / INDIA / AUSTRALIA / NEW ZEALAND / REST OF WORLD

E-mail: ads@science-int.co.uk
Fax: +44 (0) 1223 326532

Axel Gesatzki
Phone: +44 (0)1223 326529

Lucy Nelson
Phone: +44 (0)1223 326527

Kelly Grace
Phone: +44 (0) 1223 326528

JAPAN

Yuri Kobayashi
Phone: +81-(0)90-9110-1719
E-mail: ykobayas@aaas.org

CHINA / KOREA / SINGAPORE / TAIWAN / THAILAND

Ruolei Wu
Phone: +86-1367-1015-294
E-mail: rwu@aaas.org

All ads submitted for publication must comply with applicable U.S. and non-U.S. laws. *Science* reserves the right to refuse any advertisement at its sole discretion for any reason, including without limitation for offensive language or inappropriate content, and all advertising is subject to publisher approval. *Science* encourages our readers to alert us to any ads that they feel may be discriminatory or offensive.



ScienceCareers.org



University of
Zurich ^{UZH}

Vetsuisse Faculty

Professorship in Veterinary Pharmacology (open rank)

The Institute of Veterinary Pharmacology and Toxicology of the Vetsuisse Faculty University of Zurich, offers a faculty position in Veterinary Pharmacology. The position is advertised as a professorship, open rank with tenure track (Assistant or Associate or Full Professor, depending on prior achievements).

Requirements: Applicants are expected to have a degree in veterinary medicine, medicine, biology or another closely related field in the life sciences and, in addition, a PhD or habilitation or equivalent. Applicants should demonstrate a strong and promising research program in pharmacology (preferably in molecular pharmacology) and a proven record in successful acquisition of extramural funding.

Experience in teaching undergraduate and graduate students is required; teaching will be coordinated with the Professor in Toxicology and the Division of Veterinary Pharmacology and Toxicology of the Vetsuisse Faculty University of Berne. Prior mentoring of graduate students is desirable. The successful candidate is expected to contribute to the service work offered by the Institute of Pharmacology and Toxicology.

More detailed information can be obtained from the Dean's office of the Vetsuisse Faculty University of Zurich.

Ideally, applicants should be fluent in English and German, although knowledge in German can also be acquired within a reasonable time period.

Younger applicants are particularly encouraged to apply. The University of Zurich strives to increase the percentage of women in leading positions and therefore strongly encourages qualified female researchers to apply.

Application process: Applicants are kindly invited to respond **no later than May 30, 2013**, by sending the following information as a complete package in English: cover letter outlining concepts for research, teaching and service work, curriculum vitae incl. structured list of publications and extramural funding; copies of relevant examinations.

Please supply a hardcopy and one comprehensive PDF-file on CD-ROM or USB key (please, no online applications) to:

University of Zurich, Vetsuisse Faculty, Dean's Office, Ms. Silvia Kaufmann, Winterthurerstrasse 204, CH-8057 Zürich, Switzerland

e-mail: dekanat@vetadm.uzh.ch



**Are you looking for an exciting academic career opportunity
in one of the most beautiful cities in the world?**

TU Dresden - one of the German Universities of Excellence - seeks to fill up to **ten**

OPEN TOPIC TENURE TRACK PROFESSORSHIPS

These professorial positions are not targeted at specific areas of expertise or particular academic disciplines. The sole selection criteria are **scientific excellence, innovation and the potential to advance research activities at TU Dresden**. We are particularly looking for younger candidates who have already demonstrated an exceptional potential and who work in **interdisciplinary research fields**. However, applications from well-established researchers who bring exceptional added value to the portfolio of TU Dresden are also welcome. International applications are especially sought after. We particularly encourage applications from women and minorities. More information about this program as well as about the Institutional Strategy of the university can be found at <http://tu-dresden.de/exzellenz/zukunftskonzept>.

Minimum requirements are a doctoral degree and publications in leading academic journals. Appointments are possible at different academic levels, depending on previous experience. Upon positive evaluation after the initial five-year appointment, the professorship will become tenured.

In addition to their CV (including awards and other honors), the applicant should include their publication list, a summary of past and future research, their vision for teaching, details on third-party funding, and a brief statement of how the applicant would contribute towards the university's on-going ambition for international excellence.

The deadline for applications is **May 15th, 2013**. Please address all correspondence to:

**Technische Universität Dresden
The Rector
- personal/confidential -
Application Open Topic Professorship
01062 Dresden
Germany**

Alternatively, applications may be sent by email to rektor@tu-dresden.de, indicating "Application for Open Topic Professorship" in the subject line.

TU Dresden has established itself as a comprehensive university with a broad range of academic subjects and an internationally renowned research portfolio. The City of Dresden is famous for its beauty and world-class cultural and scientific environment. Details may be found at <http://home.meinestadt.de/dresden/attraktivitaets-ranking>.



There's only one

DR. SHIRLEY MALCOM



To Dr. Shirley Malcom, born and raised in the segregated South more than 65 years ago, a career based on her studies in science seemed even less likely than the launch of the Soviet's Sputnik. But with Sputnik's success, the Space Race officially started and, in an instant, brought a laser-like focus to science education and ways to deliver a proper response. Not long after, Dr. Malcom entered the picture.

Although black schools at the time received fewer dollars per student and did not have sufficient resources to maintain their labs at a level equivalent to the white schools, Dr. Malcom found her way to the University of Washington where she succeeded in obtaining a B.S. in spite of the difficulties of being an African American woman in the field of science. From there she went on to earn a Ph.D. in ecology from Penn State and held a faculty position at the University of North Carolina, Wilmington.

Dr. Malcom has served at the AAAS in multiple capacities, and is presently Head of the Directorate for Education and Human Resources Programs. Nominated by President Clinton to the National Science Board, she also held a position on his Committee of Advisors on Science and Technology. She is currently a member of the Caltech Board of Trustees, a Regent of Morgan State University, and co-chair of the Gender Advisory Board of the UN Commission on Science and Technology for Development. She has held numerous other positions of distinction and is the principal author of *The Double Bind: The Price of Being a Minority Woman in Science*.

Of her active career in science, Dr. Malcom says, "I guess I have become a poster child for taking one's science background and using that in many other ways: we ask questions; we try to understand what we find; we consider what evidence we would need to confirm or refute hypotheses. And that happens in whatever setting one finds oneself."

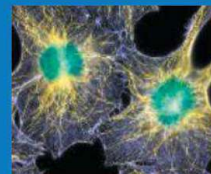
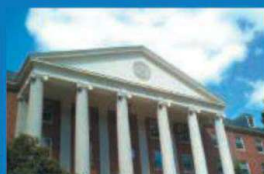
At *Science* we are here to help you in your own scientific career with expert career advice, forums, job postings, and more — all for free. Visit *Science* today at ScienceCareers.org.



For your career in science, there's only one **Science**

ScienceCareers.org

**Associate Director for Data Science
Office of the Director, National Institutes of Health, Department of Health and Human Services**



***The NIH is the center of medical and behavioral research for the Nation
----making essential medical discoveries that improve health and save lives.***

Are you a top-level Scientific Researcher or Scientific Administrator seeking a career at one of the preeminent biomedical research institutions in the Nation and the world? Are you at that point in your career where you're ready to "give back?" The position of Associate Director for Data Science (ADDS), Office of the Director (OD), National Institutes of Health (NIH), offers a unique and exciting opportunity to provide critical leadership for basic and translational research. The era of "Big Data" has arrived for the biomedical sciences. There is an urgent need and, with it, spectacular opportunities for NIH to enhance its programs in data science, such as those involving data emanating from different sources (e.g., genomics, imaging, and phenotypic information from electronic health records). The ADDS provides a vision for the utilization and extraction of knowledge from the data generated by, and relevant to, NIH research, and advises experts throughout the agency on a variety of complex, unique, and/or sensitive situations and issues in data science to ensure continual achievement of NIH's dynamic biomedical research mission.

We are looking for applicants with senior-level experience who have a commitment to excellence and the energy, enthusiasm, and innovative thinking necessary to lead a dynamic and diverse organization.

The successful candidate for this position will be appointed at a salary commensurate with his/her qualifications. Full Federal benefits will be provided including leave, health and life insurance, long-term care insurance, retirement, and savings plan (401k equivalent).

If you are ready for an exciting leadership opportunity, please see the detailed vacancy announcement at <http://www.jobs.nih.gov> (under Executive Careers). Applications will be reviewed starting May 13, 2013, and will be accepted until the position is filled.



**THE NATIONAL INSTITUTES OF HEALTH AND THE DEPARTMENT OF HEALTH AND HUMAN SERVICES
ARE EQUAL OPPORTUNITY EMPLOYERS**



ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Faculty Positions in Theoretical and Computational Neuroscience at Ecole polytechnique fédérale de Lausanne (EPFL)

The School of Life Sciences at EPFL invites applications for two positions of tenure track Assistant Professor in Computational Neuroscience. In exceptional cases of more senior profiles, appointment at the level of associate or full professor may also be considered.

The search is broad, but research areas of particular interest are:

- Machine learning tools for computational neuroscience such as data-driven modeling, data analysis, optimization of neuron models, regularization, or large-scale neuroscience data analysis
- Theory and simulation of cognitive brain processes including theories of risk and decision-making, models of memory formation or working memory, learning, attention and emotion.

Candidates with other profiles are also considered

EPFL is committed to excellence in teaching and research and provides a dynamic environment including the Brain Mind Institute of the School of Life Sciences, the School of Computer and Communication Sciences, the Center for Neuroprosthetics, as well as the recently established Neuropolis initiative, together with the Universities of Lausanne and Geneva. The successful candidate is expected to develop an independent and competitive research program in a

multidisciplinary environment and engage with researchers in related fields across the EPFL campus, as computer science, machine learning, scientific computing, neuroprosthetics, mathematical finance, human brain imaging, psychophysics, and the Human Brain Project.

Applications including letter of motivation, curriculum vitae, publication list, concise statement of research and teaching interests as well as the names and addresses (including email) of at least five references should be submitted in PDF format via the website:

<http://comp-neurosci.epfl.ch>

While the committee will start analyzing applications on **August 15th, 2013**, the search will remain open until the positions are filled.

For further inquiries you may contact **Prof. Wulfram Gerstner** (chair of search committee) at **comp-neurosci.search@epfl.ch**

For additional information, please consult **<http://sv.epfl.ch/home>** and **<http://www.epfl.ch>**

EPFL is committed to expanding the ranks of women on its faculty, and qualified women are enthusiastically encouraged to apply.



AAAS is here – helping scientists achieve career success.

Every month, over 400,000 students and scientists visit ScienceCareers.org in search of the information, advice, and opportunities they need to take the next step in their careers.

A complete career resource, free to the public, *Science* Careers offers a suite of tools and services developed specifically for scientists. With hundreds of career development articles, webinars and downloadable booklets filled with practical advice, a community forum providing answers to career questions, and thousands of job listings in academia, government, and industry, *Science* Careers has helped countless individuals prepare themselves for successful careers.

As a AAAS member, your dues help AAAS make this service freely available to the scientific community. If you're not a member, join us. Together we can make a difference.

To learn more, visit aaas.org/plusyou/sciencecareers



THE END OF CANCER BEGINS WITH YOU

ASSOCIATE CENTER DIRECTOR FOR BASIC SCIENCE

Moffitt Cancer Center is seeking applicants for a faculty appointment at the level of Associate Center Director (ACD) for Basic Science. The successful candidate will direct all Research Programs and Shared Facilities assigned to the Division of Basic Science of the Moffitt Research Institute (MRI). The ACD sets strategic direction and priorities in basic science research and is accountable for the performance and results of Basic Science; decisions have a direct impact on the MRI and Moffitt Medical Group with implications to the entire organization. The ACD maintains an active research program funded principally by extramural grants, publishes original reports in peer-reviewed journals, contributes service to professional societies and contributes to teaching and mentoring of faculty, staff, fellows and graduate students. The ACD reports to the Center Director and provides leadership and direction through Department Chairs, Program Leaders, Shared Resource Leaders, Vice Presidents, Directors and Faculty.

12902 MAGNOLIA DRIVE, TAMPA, FL 33612
1-888-MOFFITT | MOFFITT.ORG

Moffitt Cancer Center provides a tobacco-free work environment. We are an equal opportunity, affirmative action employer and a drug-free workplace.

REQUIREMENTS

Qualified applicants will have an M.D. or Ph.D. in a field relevant to cancer research, as well as 10+ years' experience in leadership in a Cancer Center and/or academic setting and in grant management. In addition, the successful candidate will have a proven ability to manage programs and supervise multi-level staff, and collaborate at a senior level with other Associate Center Directors, Vice Presidents and Managers. Preference will be given to internationally recognized researchers with active laboratories and NIH funding.

For further details and to apply, visit MOFFITT.org/Careers



CLOSER TO OUR PATIENTS.
CLOSER TO A CURE.®

Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India
<http://www.jncasr.ac.in>

Faculty positions in Advanced Materials Science and Chemistry

The Chemistry and Physics of Materials Unit and the New Chemistry Unit at the Jawaharlal Nehru Centre for Advanced Scientific Research, (JNCASR), Bangalore, invites applications from Indian nationals for the position of Faculty Fellow (equivalent to an Assistant Professor) in the fields of (a) experimental materials science (especially in energy or biomaterials) and (b) synthetic biology. Candidates must have excellent academic credentials throughout their career and are expected to develop independent, high quality research programmes.

Applicants should have a Ph.D. in chemistry/physics/related branch of engineering and a minimum of three years of postdoctoral research experience. Please send your (a) curriculum vitae, (b) two-page research plan and (c) names of five referees to:

Faculty Search, Materials and Chemistry, Jawaharlal Nehru Centre for Advanced Scientific Research
Jakkur, Bangalore-560 064, INDIA
Email: mcsearch@jncasr.ac.in



Universität Regensburg

The University Regensburg, Germany, Faculty of Medicine, invites applications for a

W 3-Professorship (Chair) in Interventional Immunology

The successful candidate will head the newly installed Department for Interventional Immunology at the Medical Faculty of the Regensburg University. The position is the initial W3-Professorship of the Regensburg Center for Interventional Immunology (www.rcii.de), an interdisciplinary research center for the development of novel immunotherapies against cancer, infections and autoimmune diseases. The successful candidate is expected to lead and transform the RCI into an independent research institute collaborating closely with the University of Regensburg and its University Hospital.

We are seeking a scientist with an excellent record in the field of transplantation immunology with a focus on cellular and molecular mechanisms of alloresponses after solid organ and/or allogeneic stem cell transplantation. Though primarily a basic research position focused on the development of innovative immunological treatment strategies in transplantation, the candidate should be able to support translational research and early clinical trials in cooperation with the respective clinical partners at the University Hospital Regensburg. Furthermore, competence for developing the RCI into an independent research institute is expected.

Required qualifications for applicants include a university degree, demonstrated teaching abilities, an M.D. or Ph.D. degree, as well as scientific achievements equivalent to the German "Habilitation". Applicants should not be beyond the age of 52 at the time of appointment. Exceptions are possible according to Article 10, Paragraph 3, Sentence 2 BayHSGPG.

The University of Regensburg is an equal opportunity employer. Applications by handicapped individuals will be prioritized.

The Regensburg University is committed to increase the percentage of female scientists and encourages female applicants to apply. Furthermore, special importance is given to improving the compatibility of family and career (for further information see <http://chancengleichheitfamilie.uni-regensburg.de>).

Please submit your application with your curriculum vitae, certificates, diplomas, list of publications including reprints of the 10 most significant reprints, your extramural funding record and a precise survey of your teaching experience by **May 31, 2013** to the **Dean of the Faculty of Medicine at the University of Regensburg, Franz-Josef-Strauß-Allee 11, 93053 Regensburg, Germany.**

Please send a written application and please use the application form at: <http://www.uni-regensburg.de/Fakultaeten/Medizin/index.html>

There's only one GALILEO GALILEI

Born in 1564, Galileo Galilei once contemplated a career in the priesthood. It's perhaps fortunate for science that upon the urging of his father, he instead decided to enroll at the University of Pisa. His career in science began with medicine and from there he subsequently went on to become a philosopher, physicist, mathematician, and astronomer, for which he is perhaps best known. His astronomical observations and subsequent improvements to telescopes built his reputation as a leading scientist of his time, but also led him to probe subject matter counter to prevailing dogma. His expressed views on the Earth's movement around the sun caused him to be declared suspect of heresy, which for some time led to a ban on the reprinting of his works.

Galileo's career changed science for all of us and he was without doubt a leading light in the scientific revolution, which is perhaps why Albert Einstein called him the father of modern science.

Want to challenge the status quo and make the Earth move? At *Science* we are here to help you in your own scientific career with expert career advice, forums, job postings, and more — all for free. For your career in science, there's only one *Science*. Visit ScienceCareers.org today.

For your career in science, there's only one **Science**

ScienceCareers.org





The EGL Charitable Foundation
invites you to apply to the
**Gruss Lipper Post-Doctoral
Fellowship Program**

Eligibility

- Israeli citizenship
- Candidates must have completed PhD and/or MD/PhD degrees in the Biomedical Sciences at an accredited Israeli University/Medical School or be in their final year of study
- Candidates must have been awarded a postdoctoral position in the U.S. host research institution.

**Details regarding the fellowship are available
at www.eglcdf.org**

NW210508R

Science Careers is the forum
that answers questions.



Science Careers is dedicated to opening new doors and providing timely answers to the career questions that matter to you.

Science Careers Forum:

- » Relevant Career Topics
- » Timely Advice and Answers
- » Community, Connections, and More!

Visit the forum and join
the conversation today!

Your Future Awaits.



Universitätsklinikum Würzburg



The **Interdisciplinary Center for Clinical Research (IZKF)** organizes the internal research funding of the Medical Faculty of the University of Würzburg. Its major goal is to strengthen clinical research on the basis of interdisciplinary cooperation between clinical and basic research groups. To carry out its mission, the IZKF supports cooperative research grants, promotes training and advancement of young researchers in medicine and improves the scientific infrastructure. For more information see www.izkf.uk-wuerzburg.de.

The IZKF intends to establish a new

Junior Research Group Infection Biology of Human Pathogens

to be affiliated with the **Research Center for Infectious Diseases (ZINF)**. We are looking for a researcher with outstanding postdoctoral experience and international recognition in the general fields of microbiology, cell biology, mycology, parasitology, immunology, or host-pathogen interactions. Candidates investigating the biology of clinically relevant human pathogens, in particular pathogenic fungi or bacteria are preferred, but all outstanding infection biologists are encouraged to apply. Interactions with other groups and members of the ZINF, the IZKF as well as with clinical partners are expected.

In addition to the **position of the Group Leader** the grant of the IZKF will provide for up to 5 years funding for:

- a postdoctoral scientist
- a PhD student
- a technician
- Consumables: approx. 50.000 Euro/year
- Equipment: approx. 50.000 Euro start-up financing

Laboratory space and basic equipment will be provided at the Institute for Molecular Infection Biology (IMIB; www.imib-wuerzburg.de) in a new 9,000 m² state-of-the-art research building with bio-containment, molecular imaging, high-throughput genomics, and animal facilities. The building is specifically designed to foster collaborative research, and is home to several additional young investigator groups as well as laboratories run by faculty members. See www.imib-wuerzburg.de for more information.

Interested individuals should send a one-page description of their research interests and future directions, CV and publication list, and the names of three academic referees by **May 15, 2013**. We may request that short-listed candidates provide a more detailed research proposal at a later date. These candidates also will be invited to present their research in Würzburg on June 28-29, 2013.

Preference will be given to people with disabilities in the case of otherwise equal aptitude. The University aims to increase the proportion of female researchers, therefore applications from qualified women are particularly welcome. Applications should be sent via email in one pdf-file to the IZKF office (hawks_m@klinik.uni-wuerzburg.de). Informal inquiries can be made to Prof. Jörg Vogel (joerg.vogel@uni-wuerzburg.de).

IZKF Würzburg
Dr. Andrea Thelen-Frölich
Josef-Schneider-Str.2 · 97080 Würzburg
Telefax: ++49(0)931 201-47414
Email: Hawks_M@klinik.uni-wuerzburg.de
Internet: www.izkf.uk-wuerzburg.de



AAAS is here – promoting universal science literacy.

In 1985, AAAS founded Project 2061 with the goal of helping all Americans become literate in science, mathematics, and technology. With its landmark publications *Science for All Americans* and *Benchmarks for Science Literacy*, Project 2061 set out recommendations for what all students should know and be able to do in science, mathematics, and technology by the time they graduate from high school. Today, many of the state standards in the United States have drawn their content from Project 2061.

Every day Project 2061 staff use their expertise as teachers, researchers, and scientists to evaluate textbooks and assessments, create conceptual strand maps for educators, produce groundbreaking research and innovative books, CD-ROMs, and professional development workshops for educators, all in the service of achieving our goal of universal science literacy.

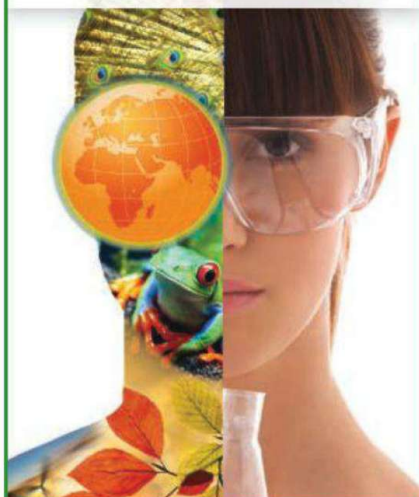
As a AAAS member, your dues help support Project 2061 as it works to improve science education. If you are not yet a AAAS member, join us. Together we can make a difference.

To learn more, visit aaas.org/plusyou/project2061



WOMEN IN SCIENCE

forging
new pathways in
green
science



Read inspiring stories
of women working in
"Green Science"
who are blending
a unique combination of
enthusiasm for science
and concern for others
to make the world
a better place.

Download this
free booklet
[ScienceCareers.org/
LOrealWiS](http://ScienceCareers.org/LOrealWiS)



This booklet is brought to you by the
AAAS/Science Business Office
in partnership with the
L'Oreal Foundation

PRIZES



Ministry of Science
and Technology



Israel Prime Minister's Office



Keren Hayesod
United Israel Appeal

Call for nominations for the first annual Eric and Sheila Samson Prime Minister's Prize for Innovation in Alternative Fuels for Transportation

The US \$1 million prize will be awarded for global innovation or a major scientific or technological breakthrough in the field of alternative fuels for transportation.

Nominees for the prize should be actively engaged in innovative, paradigm-shifting research in the field of alternative fuels for transportation, worthy of significant and widespread attention. The recipient(s) can be of any nationality, race, ethnicity, or sex.

Deadline for nominations: May 31, 2013

For nomination information and forms see
<http://tinyurl.com/pmprize>

POSITIONS OPEN



3 Senior Faculty Positions Cryo Electron Microscopy, X-ray Crystallography, and NMR Sealy Center for Structural Biology and Molecular Biophysics

UTMB Health seeks senior faculty applicants in structural biology in the Sealy Center for Structural Biology and Molecular Biophysics (SCSBMB). The Center supports a graduate program in molecular biophysics and five well-run core laboratories in Cryo-EM, NMR, X-ray, Computation and Solution Biophysics, each with an excellent PhD-level manager. For details see: <http://www.scsb.utmb.edu/>

The successful candidate must be a highly motivated individual with a PhD or MD degree, a strong publication record, and a record of independent well-funded grant support. The ideal candidate will have extensive experience in their specialty applied to the study of the structure, novel mechanisms, and functions of bio-macromolecules. Candidates for positions in either the department of Biochemistry and Molecular Biology or Pharmacology and Toxicology should also have or seek overlap with the highly collaborative established biomedical research community in UTMB's basic science departments, centers and programs of excellence such as the Institute for Human Infection and Immunity, the Galveston National Laboratory, the Center for Tropical Diseases, the Institute for Translational Sciences, the Sealy Center for Cancer Cell Biology, the Sealy Center for Environmental Health and Medicine, the Sealy Center on Aging, the George P. and Cynthia Woods Mitchell Center for Neurodegenerative Diseases, the Moody Center for Brain and Spinal Cord Injury Research, the Sealy Center for Molecular Medicine and the Chemical Biology Program. These and other entities provide a wide variety of core services, in recombinant DNA, genomics, proteomics, high-throughput drug screening, mass spectrometry, membrane protein crystallization, and protein expression and purification. Excellent collaborative opportunities also exist through UTMB's participation in the Gulf Coast Consortia and the Keck Center for Interdisciplinary Bioscience, <http://www.gulfcoastconsortia.org/home.aspx>.

Applicants are requested to submit electronically: a cover letter expressing interest in being considered, a curriculum vitae, current funding, a summary of research accomplishments, and future goals to mail to: SCSBMB.recruiting@UTMB.edu. Direct inquiries to **Dr. B. Montgomery Pettitt**, mpettitt@utmb.edu, 409-772-0723.

The University of Texas Medical Branch is an Affirmative Action/Equal Opportunity Employer.



POSTDOCTORAL POSITIONS

The Center of Excellence in Environmental Toxicology (CEET) at the University of Pennsylvania Perelman School of Medicine announces the availability of two postdoctoral positions on its Translational Research Training Program in Environmental Health Sciences. The focus of the Center includes research in Oxidative Stress/Oxidative Stress Injury; Reproduction, Endocrinology, and Development (READ); Lung and Airway Disease; and Gene-Environment Interactions.

Postdoctoral applicants must conduct full-time research in translational environmental health sciences. Must be U.S. Citizen or Permanent Resident and would be supported for up to two years. Salary is commensurate with NRSA approved levels. For further information, application procedure, and list of faculty mentors, please go to the CEET Website. The deadline to apply for the 2013–2014 term is Friday, May 24, 2013.

DIRECTOR OF BUSINESS DEVELOPMENT

The Fraunhofer Center for Molecular Biotechnology (CMB) in Newark, Delaware has an immediate opening for a Director of Business Development. The successful candidate will design and implement a comprehensive strategy to identify potential collaborators that can utilize CMB's unique plant-based protein production platform technology to expedite product development. CMB's technology has applications in the development of vaccines, therapeutics, and diagnostics. Desired qualifications include a Master's or Ph.D. in a biological discipline, an MBA and demonstrated success in business development, grant writing and collaboration with business, government, and not-for-profit research and development funding entities. The successful applicant will be highly motivated, have a high energy, positive, "can-do" attitude and a high degree of initiative. *FhCMB offers competitive salaries and benefit packages and is an Equal Opportunity Employer.* Interested candidates should send their resume or curriculum vitae to e-mail: dervin@fraunhofer-cmb.org.

DIRECTOR

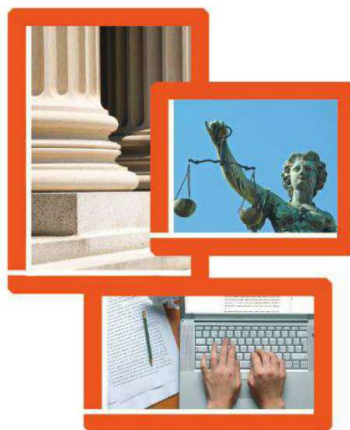
Princeton University Department of Chemistry seeks candidates for the position of Director of the new Small Molecule Screening Center.

The director will provide leadership in managing program activities and will be responsible for the overall operations of the screening center by employing a wide range of contemporary high throughput screening assays; performance of all follow-up organic synthesis activities; and, maintenance of the screening center infrastructure.

The successful candidate must hold a Ph.D. in organic synthesis (or related field) and have broad experience and knowledge in medicinal chemistry. The candidate should have current bench skills, demonstrated proficiency in mass spectroscopy technology and analytical chemistry. For further information and to apply online (required) at website: <https://jobs.princeton.edu> (requisition #1300162). Submit a cover letter, curriculum vitae, list of publications and contact information for three references. *Princeton University is an Equal Opportunity Employer and complies with applicable EEO and Affirmative Action regulations.*

NASA BIOSIGNATURES INVESTIGATOR

NASA Ames Research Center in the San Francisco Bay Area is seeking a Biosignatures Principal Investigator to join its Exobiology Branch. The position is responsible for conducting microbiological and/or geological research to identify biosignatures that can reveal and characterize past or present life in environments relevant to astrobiology, such as Mars. A job announcement, required qualifications, and application procedure are available on website: <https://www.usajobs.gov/> by searching for announcement #A13B0008.



Nontraditional Careers: Opportunities Away From the Bench Webinar

Want to learn more about exciting and rewarding careers outside of academic/industrial research? View a roundtable discussion that looks at the various career options open to scientists and strategies you can use to pursue a nonresearch career.

Now Available On Demand

www.sciencecareers.org/webinar

Produced by the
Science/AAAS Business Office.



Your career is our cause.

Get help from the experts.

www.sciencecareers.org

- Job Postings
- Job Alerts
- Resume/CV Database
- Career Advice
- Career Forum

Science Careers

From the journal Science AAAS

Find your future here.



Science Careers

From the journal Science AAAS

www.ScienceCareers.org

MARKETPLACE

ProMab Biotechnologies, Inc.

Custom Monoclonal Antibody \$3,900

> 3,000 CLONES WILL BE SCREENED

1-866-339-0871

www.ProMab.com | info@promab.com

Congratulations to the 2013 AAAS Student Poster Competition Winners

The 2013 Student Poster Competition took place at the AAAS Annual Meeting in Boston 14-18 February. The student winners' work displayed originality and understanding that set them apart from their peers.

BRAIN AND BEHAVIOR

Winner: Michelle Oberoi,
University of California, Irvine
Parvalbumin Basket Cells Preferentially Select Hippocampal CA1 Pyramidal Cells

Honorable Mention: Yakov Kronrod,
University of Maryland and Emily Coppess, University of Chicago
A Unified Model of Categorical Effects in Consonant and Vowel Perception

CELLULAR AND MOLECULAR BIOLOGY

Winner: Ricardo Ramirez,
University of California, Irvine
Dynamics of Transcriptional Regulation and Chromatin Accessibility During Myeloid Differentiation

Honorable Mention: Robert Welliver and Joseph Modica, Canisius College
Promotion of Microtubule Acetylation Inhibits Growth Cone Advance in Regenerating Axons

DEVELOPMENTAL BIOLOGY, PHYSIOLOGY, AND IMMUNOLOGY

Winner: Stone Jiang,
California Institute of Technology
Engineering Novel Antibody-Like Reagents to Improve Neutralization of HIV

Honorable Mention: Le Chang, Greg Astrian, and Choon Chung,
University of California, Los Angeles
Human Perivascular Stem Cells Induce Bone Regeneration in a Rat Spinal Fusion Model

ENVIRONMENT AND ECOLOGY

Winner: Sean Kent,
Northeastern University
The Importance of Ecological Context: The Effect of Neighboring Exotic Plants on the Herbivory of Common Milkweed by the Monarch Butterfly

Honorable Mention: Amanda M. Franklin, University of Melbourne
Costs of Copulation in a Promiscuous Cephalopod

MATH, TECHNOLOGY, AND ENGINEERING

Winner: Christopher Luna, Arizona State University
Direct Numerical Simulations of Phytoplankton Blooms

Honorable Mention: Zachariah J. Berkson, Arizona State University
EPR Study of Charge Separation within Oxygen-Deficient TiO₂ Photocatalysts for the Conversion of Carbon Dioxide to Fuels

MEDICINE AND PUBLIC HEALTH

Winner: Elizabeth Brito, Arizona State University
The Effect of a Community-Based Diabetes Prevention Program on Weight-Specific Quality of Life in Latino Adolescents

Honorable Mention: Kristin A. Linn, North Carolina State University
Interactive Q-Learning for Dynamic Treatment Regimes

PHYSICAL SCIENCES

Winner: Philip S. Burnham, Villanova University
Comparative Study of Surfactant-Coated Ferromagnetic Nanoparticles for Hyperthermia Application

Honorable Mention: Toni F. Martin, George Mason University
Conversion of Bicarbonate to Carbonate as a Function of Time and Temperature

SOCIAL SCIENCES

Winner: Meredith T. Niles, University of California, Davis
Climate Change Policy Adaptation Among California Farmers: How Policy Experience and Attitudes Affect Climate Change Perceptions and Behaviors

Honorable Mention: Rider W. Foley, Arizona State University
Comparing Cultures of Technological Innovation: Risk Versus Reward

 **AAAS|2014**
ANNUAL MEETING
13-17 FEBRUARY • CHICAGO

www.aaas.org/meetings/2014

The 2014 poster entry site opens on **14 May 2013**.

The AAAS Student Poster Competition is open to students actively working toward an undergraduate, graduate, or doctoral degree. Winners receive a cash award, certificate, and recognition in the journal *Science*. Individuals holding doctoral degrees are invited to participate in the General Poster session.